

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau(43) International Publication Date
15 January 2004 (15.01.2004)

PCT

(10) International Publication Number
WO 2004/005277 A1(51) International Patent Classification?: C07D 333/32,
A61K 31/38TOWNSEND, Craig, A. [US/US]; Department of Chem-
istry, John Hopkins University, 3400 N. Charles Street,
Baltimore, MD 21218 (US).(21) International Application Number:
PCT/US2003/021700(74) Agents: STIEFEL, Maurice, B. et al.; Bryan Cave LLP,
1290 Avenue of the Americas, New York, NY 10167 (US).

(22) International Filing Date: 9 July 2003 (09.07.2003)

(25) Filing Language: English

(26) Publication Language: English

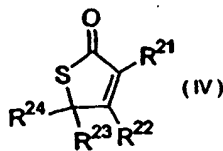
(30) Priority Data:
60/394,585 9 July 2002 (09.07.2002) US(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VC, VN, YU, ZA, ZM, ZW.(71) Applicants (for all designated States except US): FAS-
GEN, INC. [US/US]; Bayview Medical Campus, 5210
Eastern Avenue, Baltimore, MD 21224 (US). THE
JOHNS HOPKINS UNIVERSITY [US/US]; 4910
Eastern Avenue, Baltimore, MD 21224 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KUHAJDA,
Francis, P. [US/US]; John Hopkins Medical Center,
4940 Eastern Avenue, Baltimore, MD 21224 (US).
MEDGHALCHI, Susan, M. [US/US]; Fasgen, Inc. Uni-
versity, Johns Hopkins Bayview Campus, 5210 Eastern
Avenue, Baltimore, MD 21224 (US). MCFADDEN, Jill,
M. [US/US]; The Johns Hopkins University, Departments
of Chemistry, 3400 N. Charles Street, Baltimore, MD
21218 (US). THUPARI, Jagan [US/US]; Department
of Pathology, John Hopkins Bayview Research Cam-
pus, 5210 Eastern Avenue, Baltimore, MD 21224 (US).(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.(54) Title: NOVEL COMPOUNDS, PHARMACEUTICAL COMPOSITIONS CONTAINING SAME, AND METHODS OF USE
FOR SAME(57) Abstract: A pharmaceutical composition comprising a pharmaceutical diluent and a
compound of formula IV wherein R²¹ = H, C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or
alkylaryl, -CH₂OR²⁵, -C(O)R²⁵, -CO(O)R²⁵, -C(O)NR²⁵R²⁶, -CH₂C(O)R²⁵, or -CH₂C(O)NHR²⁵,
where R₂₅ and R₂₆ are each independently H, C₁-C₁₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or
alkylaryl, optionally containing one or more halogen atoms. R²² = -OH, -OR²⁷, -OCH₂C(O)R²⁷,
-OCH₂C(O)NHR²⁷, -OC(O)R²⁷, -OC(O)OR²⁷, -OC(O)NHNH-R³, or -OC(O)NR²⁷R²⁸, where R²⁷
and R²⁸ are each independently H, C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, andwhere R²⁷ and R²⁸ can each optionally contain halogen atoms; R²³ and R²⁴, the same or different from each other, are C₁-C₂₀ alkyl,
cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl. Methods of using such formulations for the treatment of cancer, to effect weight
loss, to treat microbially-based infections, to inhibit neuropeptide-Y and/or fatty acid synthase, and to stimulate CPT-1.

12/pst

NOVEL COMPOUNDS, PHARMACEUTICAL COMPOSITIONS CONTAINING SAME, AND METHODS OF USE FOR SAME

BACKGROUND OF THE INVENTION

5 Fatty acid synthase

Fatty acids have three primary roles in the physiology of cells. First, they are the building blocks of biological membranes. Second, fatty acid derivatives serve as hormones and intracellular messengers. Third, and of particular importance to the present invention, fatty acids are fuel molecules that can be stored in adipose tissue as triacylglycerols, which are also known
10 as neutral fats.

There are four primary enzymes involved in the fatty acid synthetic pathway, fatty acid synthase (FAS), alkynyl CoA carboxylase (ACC), malic enzyme, and citric lyase. The principal enzyme, FAS, catalyzes the NADPH-dependent condensation of the precursors malonyl-CoA and alkynyl-CoA to produce fatty acids. NADPH is a reducing agent that
15 generally serves as the essential electron donor at two points in the reaction cycle of FAS. The other three enzymes (*i.e.*, ACC, malic enzyme, and citric lyase) produce the necessary precursors. Other enzymes, for example the enzymes that produce NADPH, are also involved in fatty acid synthesis.

FAS has an Enzyme Commission (E.C.) No. 2.3.1.85 and is also known as fatty
20 acid synthetase, fatty acid ligase, as well as its systematic name acyl-CoA:malonyl-CoA C-acyltransferase (decarboxylating, oxoacyl- and enoyl-reducing and thioester-hydrolysing). There are seven distinct enzymes – or catalytic domains - involved in the FAS catalyzed synthesis of fatty acids: alkynyl transacylase, malonyl transacylase, beta-ketoacyl synthetase (condensing enzyme), beta-ketoacyl reductase, beta-hydroxyacyl dehydrase, enoyl reductase, and thioesterase.

(Wakil, S. J., Biochemistry, 28: 4523-4530, 1989). All seven of these enzymes together form FAS.

Although the FAS catalyzed synthesis of fatty acids is similar in lower organisms, such as, for example, bacteria, and in higher organisms, such as, for example, mycobacteria, yeast and humans, there are some important differences. In bacteria, the seven enzymatic reactions are carried out by seven separate polypeptides that are non-associated. This is classified as Type II FAS. In contrast, the enzymatic reactions in mycobacteria, yeast and humans are carried out by multifunctional polypeptides. For example, yeast have a complex composed of two separate polypeptides whereas in mycobacterium and humans, all seven reactions are carried out by a single polypeptide. These are classified as Type I FAS.

FAS inhibitors

Various compounds have been shown to inhibit fatty acid synthase (FAS). FAS inhibitors can be identified by the ability of a compound to inhibit the enzymatic activity of purified FAS. FAS activity can be assayed by measuring the incorporation of radiolabeled precursor (i.e., alkynyl-CoA or malonyl-CoA) into fatty acids or by spectrophotometrically measuring the oxidation of NADPH. (Dils, et al., Methods Enzymol., 35:74-83).

Table 1, set forth below, lists several FAS inhibitors.

Table 1**Representative Inhibitors Of The Enzymes Of The Fatty Acid Synthesis Pathway****Inhibitors of Fatty Acid Synthase**

1,3-dibromopropanone
 Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid), DTNB)
 4-(4'-chlorobenzoyloxy) benzyl nicotinate (KCD-232)
 4-(4'-chlorobenzoyloxy) benzoic acid (MII)
 2(5(4-chlorophenyl)pentyl)oxirane-2-carboxylate (POCA) and its CoA derivative
 ethoxyformic anhydride

cerulenin
 phenocerulein
 melarsoprol
 iodoacetate
 phenylarsineoxide
 pentostam
 melittin
 thiolactomycin

Inhibitors for citrate lyase

(-) hydroxycitrate
 (R,S)-S-(3,4-dicarboxy-3-hydroxy-3-methyl-butyl)-CoA
 S-carboxymethyl-CoA

Inhibitors for malic enzyme

periodate-oxidized 3-aminopyridine adenine
 dinucleotide phosphate
 5,5'-dithiobis(2-nitrobenzoic acid)
 p-hydroxymercuribenzoate
 N-ethylmaleimide
 oxalyl thiol esters such as S-oxalylglutathione
 gossypol
 phenylglyoxal
 2,3-butanedione
 bromopyruvate
 pregnenolone

Inhibitors for alkynyl CoA carboxylase

sethoxydim
 haloxyfop and its CoA ester
 diclofop and its CoA ester
 clethodim
 alloxydim
 trifop
 clofibric acid
 2,4-D mecoprop
 dalapon
 2-alkyl glutarate
 2-tetradecanylethylglutarate (TDG)
 2-octylethylglutaric acid
 N6,02-dibutyryl adenosine cyclic 3',5'-monophosphate
 N2,02-dibutyryl guanosine cyclic 3',5'-monophosphate
 CoA derivative of 5-(tetradecyloxy)-2-furoic acid (TOFA)
 2,3,7,8-tetrachlorodibenzo-p-dioxin

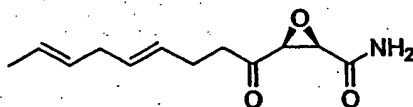
9-decenyl-1-pentenedioic acid
 decanylethyl-2-pentenedioic acid
 decanylethyl-1-pentenedioic acid
 (S)-ibuprofenyl-CoA
 (R)-ibuprofenyl-CoA
 fluazifop and its CoA ester
 clofop
 5-(tetradecyloxy)-2-furoic acid
 beta, beta'-tetramethylhexadecanedioic acid
 tralkoxydim
 free or monothioester of beta, beta prime-methyl-substituted hexadecanedioic acid (MEDICA 16)
 alpha-cyano-4-hydroxycinnamate
 S-(4-bromo-2,3-dioxobutyl)-CoA
 p-hydroxymercuribenzoate (PHMB)
 N6,02-dibutyryl adenosine cyclic 3',5'-monophosphate

Of the four enzymes in the fatty acid synthetic pathway, FAS is the preferred target for inhibition because it acts only within the pathway to fatty acids, while the other three enzymes are implicated in other cellular functions. Therefore, inhibition of one of the other three enzymes is more likely to affect normal cells. Of the seven enzymatic steps carried out by FAS, the step catalyzed by the condensing enzyme (*i.e.*, beta-ketoacyl synthetase) and the enoyl reductase have been the most common candidates for inhibitors that reduce or stop fatty acid synthesis. The condensing enzyme of the FAS complex is well characterized in terms of structure and function. The active site of the condensing enzyme contains a critical cysteine thiol, which is the target of antilipidemic reagents, such as, for example, the inhibitor cerulenin.

Preferred inhibitors of the condensing enzyme include a wide range of chemical compounds, including alkylating agents, oxidants, and reagents capable of undergoing disulphide exchange. The binding pocket of the enzyme prefers long chain, *E, E*, dienes.

In principal, a reagent containing the sidechain diene and a group which exhibits reactivity with thiolate anions could be a good inhibitor of the condensing enzyme. Cerulenin

[(2*S*, 3*R*)-2,3-epoxy-4-oxo-7,10 dodecadienoyl amide] is an example:



Cerulenin covalently binds to the critical cysteine thiol group in the active site of the condensing enzyme of fatty acid synthase, inactivating this key enzymatic step (Funabashi, et al., J. Biochem., 105:751-755, 1989). While cerulenin has been noted to possess other activities, these either occur in microorganisms which may not be relevant models of human cells (*e.g.*, inhibition of cholesterol synthesis in fungi, Omura (1976), Bacteriol. Rev., 40:681-697; or diminished RNA synthesis in viruses, Perez, et al. (1991), FEBS, 280: 129-133), occur at a substantially higher

drug concentrations (inhibition of viral HIV protease at 5 mg/ml, Moelling, et al. (1990), FEBS, 261:373-377) or may be the direct result of the inhibition of endogenous fatty acid synthesis (inhibition of antigen processing in B lymphocytes and macrophages, Falo, et al. (1987), J. Immunol., 139:3918-3923). Some data suggest that cerulenin does not specifically inhibit
5 myristoylation of proteins (Simon, et al., J. Biol. Chem., 267:3922-3931, 1992).

Several more FAS inhibitors are disclosed in U.S. Patent Application No. 08/096,908 and its CIP filed Jan. 24, 1994, the disclosures of which are hereby incorporated by reference. Included are inhibitors of fatty acid synthase, citrate lyase, CoA carboxylase, and malic enzyme.

10 Tomoda and colleagues (Tomoda et al., Biochim. Biophys. Act 921:595-598 1987; Omura et al., J. Antibiotics 39:1211-1218 1986) describe Triacsin C (sometimes termed WS-1228A), a naturally occurring acyl-CoA synthetase inhibitor, which is a product of *Streptomyces* sp. SK-1894. The chemical structure of Triacsin C is 1-hydroxy-3-(E, E, E-2',4',7'-undecatrienyldine) triazene. Triacsin C causes 50% inhibition of rat liver acyl-CoA synthetase at
15 8.7 μ M; a related compound, Triacsin A, inhibits acyl CoA-synthetase by a mechanism which is competitive with long-chain fatty acids. Inhibition of acyl-CoA synthetase is toxic to animal cells. Tomoda et al. (Tomoda et al., J. Biol. Chem. 266:4214-4219, 1991) teaches that Triacsin C causes growth inhibition in Raji cells at 1.0 μ M, and have also been shown to inhibit growth of Vero and Hela cells. Tomoda et al. further teaches that acyl-CoA synthetase is essential in
20 animal cells and that inhibition of the enzyme has lethal effects.

A family of compounds (gamma-substituted-alpha-methylene-beta-carboxy-gamma-butyrolactones) has been shown in U.S. Patent No. 5,981,575 (the disclosure of which is hereby incorporated by reference) to inhibit fatty acid synthesis, inhibit growth of tumor cells,

and induce weight loss. The compounds disclosed in the '575 Patent have several advantages over the natural product cerulenin for therapeutic applications: [1] they do not contain the highly reactive epoxide group of cerulenin, [2] they are stable and soluble in aqueous solution, [3] they can be produced by a two-step synthetic reaction and thus easily produced in large quantities, and [4] they are easily tritiated to high specific activity for biochemical and pharmacological analyses. The synthesis of this family of compounds, which are fatty acid synthase inhibitors, is described in the '575 Patent, as is their use as a means to treat tumor cells expressing FAS, and their use as a means to reduce body weight. The '575 Patent also discloses the use of any fatty acid synthase inhibitors to systematically reduce adipocyte mass (adipocyte cell number or size) as a means to reduce body weight.

The primary sites for fatty acid synthesis in mice and humans are the liver (*see* Roncari, *Can. J. Biochem.*, 52:221-230, 1974; Triscari et al., 1985, *Metabolism*, 34:580-7; Barakat et al., 1991, *Metabolism*, 40:280-5), lactating mammary glands (*see* Thompson, et al., *Pediatr. Res.*, 19:139-143, 1985) and adipose tissue (Goldrick et al., 1974, *Clin. Sci. Mol. Med.*, 46:469-79).

Inhibitors of fatty acid synthesis as antimicrobial agents

Cerulenin was originally isolated as a potential antifungal antibiotic from the culture-broth of *Cephalosporium caerulens*. Structurally cerulenin has been characterized as (2*R*,3*S*)-epoxy-4-oxo-7,10-trans,trans-dodecanoic acid amide. Its mechanism of action has been shown to be inhibition, through irreversible binding, of beta-ketoacyl-ACP synthase, the condensing enzyme required for the biosynthesis of fatty acids. Cerulenin has been categorized as an antifungal, primarily against *Candida* and *Saccharomyces sp.* In addition, some in vitro activity has been shown against some bacteria, actinomycetes, and mycobacteria, although no

activity was found against *Mycobacterium tuberculosis*. The activity of fatty acid synthesis inhibitors and cerulenin in particular has not been evaluated against protozoa such as *Toxoplasma gondii* or other infectious eucaryotic pathogens such as *Pneumocystis carinii*, *Giardia lamblia*, *Plasmodium sp.*, *Trichomonas vaginalis*, *Cryptosporidium*, *Trypanosoma*,
5 *Leishmania*, and *Schistosoma*.

Infectious diseases which are particularly susceptible to treatment are diseases which cause lesions in externally accessible surfaces of the infected animal. Externally accessible surfaces include all surfaces that may be reached by non-invasive means (without cutting or puncturing the skin), including the skin surface itself, mucus membranes, such as those covering
10 nasal, oral, gastrointestinal, or urogenital surfaces, and pulmonary surfaces, such as the alveolar sacs. Susceptible diseases include: (1) cutaneous mycoses or tineas, especially if caused by *Microsporum*, *Trichophyton*, *Epidermophyton*, or *Mucocutaneous candidiasis*; (2) mucotic keratitis, especially if caused by *Aspergillus*, *Fusarium* or *Candida*; (3) amoebic keratitis, especially if caused by *Acanthamoeba*; (4) gastrointestinal disease, especially if caused by
15 *Giardia lamblia*, *Entamoeba*, *Cryptosporidium*, *Microsporidium*, or *Candida* (most commonly in immunocompromised animals); (5) urogenital infection, especially if caused by *Candida albicans* or *Trichomonas vaginalis*; and (6) pulmonary disease, especially if caused by *Mycobacterium tuberculosis*, *Aspergillus*, or *Pneumocystis carinii*. Infectious organisms that are susceptible to treatment with fatty acid synthesis inhibitors include *Mycobacterium tuberculosis*,
20 especially multiply-drug resistant strains, and protozoa such as *Toxoplasma*.

Any compound that inhibits fatty acid synthesis may be used to inhibit microbial cell growth. However, compounds administered to a patient must not be equally toxic to both

patient and the target microbial cells. Accordingly, it is beneficial to select inhibitors that only, or predominantly, affect target microbial cells.

Eukaryotic microbial cells which are dependent on their own endogenously synthesized fatty acid will express Type I FAS. This is shown both by the fact that FAS inhibitors are growth inhibitory and by the fact that exogenously added fatty acids can protect normal patient cells but not these microbial cells from FAS inhibitors. Therefore, agents which prevent synthesis of fatty acids by the cell may be used to treat infections. In eukaryotes, fatty acids are synthesized by Type I FAS using the substrates alkynyl CoA, malonyl CoA and NADPH. Thus, other enzymes which can feed substrates into this pathway may also effect the rate of fatty acid synthesis and thus be important in microbes that depend on endogenously synthesized fatty acid. Inhibition of the expression or activity of any of these enzymes will effect growth of the microbial cells that are dependent upon endogenously synthesized fatty acid.

The product of Type I FAS differs in various organisms. For example, in the fungus *S. cerevisiae* the products are predominately palmitate and stearate esterified to coenzyme-A. In *Mycobacterium smegmatis*, the products are saturated fatty acid CoA esters ranging in length from 16 to 24 carbons. These lipids are often further processed to fulfill the cells need for various lipid components.

Inhibition of key steps in down-stream processing or utilization of fatty acids may be expected to inhibit cell function, whether the cell depends on endogenous fatty acid or utilizes fatty acid supplied from outside the cell, and so inhibitors of these down-stream steps may not be sufficiently selective for microbial cells that depend on endogenous fatty acid. However, it has been discovered that administration of Type I fatty acid synthesis inhibitor to such microbes makes them more sensitive to inhibition by inhibitors of down-stream fatty acid processing

and/or utilization. Because of this synergy, administration of a fatty acid synthesis inhibitor in combination with one or more inhibitors of down-stream steps in lipid biosynthesis and/or utilization will selectively affect microbial cells that depend on endogenously synthesized fatty acid. Preferred combinations include an inhibitor of FAS and alkynyl CoA carboxylase, or FAS
5 and an inhibitor of MAS.

When it has been determined that a mammal is infected with cells of an organism which expresses Type I FAS, or if FAS has been found in a biological fluid from a patient, the mammal or patient may be treated by administering a fatty acid synthesis inhibitor (Pat No. 5,614,551).

10 The inhibition of neuropeptide-Y to depress appetite and stimulate weight loss is described in International Patent Application No. PCT/US01/05316 the disclosure of which is hereby incorporated by reference. That application, however, does not describe or disclose any of the compounds disclosed in the present application

The stimulation of carnitine palmitoyl transferase-1 (CPT-1) to stimulate weight
15 loss is described in U.S. Patent Application Serial No. 60/354,480, the disclosure of which is hereby incorporated by reference. That application does not describe or disclose any of the compounds disclosed herein, either.

The use of FAS inhibitors to inhibit the growth of cancer cells is described in U.S. Patent No. 5,759,837, the disclosure of which is hereby incorporated by reference. That
20 application does not describe or disclose any of the compounds disclosed herein.

The use of FAS inhibitors to inhibit the growth of cancer cells is described in U.S. Patent No. 5,759,837, the disclosure of which is hereby incorporated by reference. That application does not describe or disclose any of the compounds disclosed herein.

Summary of the Invention

New classes of compounds have been discovered which have a variety of therapeutically valuable properties, eg. FAS-inhibition, NPY-inhibition, CPT-1 stimulation, ability to induce weight loss, and anti-cancer and anti-microbial properties.

5 It is a further object of this invention to provide a method of inducing weight loss in animals and humans by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV..

 It is a further object of the invention to provide a method of stimulating the activity of CPT-1 by administering to humans or animals a pharmaceutical composition
10 comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV.

 It is a further object of the invention to provide a method of inhibiting the synthesis of neuropeptide Y in humans or animals by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV.

 It is a further object of the invention to provide a method of inhibiting fatty acid
15 synthase activity in humans or animals by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV.

 It is a further object of this invention to provide a method of treating cancer in animals and humans by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV.

20 It is still a further object of this invention to provide a method of preventing the growth of cancer cells in animals and humans by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV.

It is a further object of this invention to provide a method of inhibiting growth of invasive microbial cells by administering a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV.

5 **Brief Description of the Drawings**

FIG. 1 shows a synthetic scheme to make thiolactamycin.

FIG. 2 shows a synthetic scheme to make certain compounds according to the invention.

10 FIG. 3 shows a synthetic scheme to make certain compounds according to the invention.

FIG. 4 shows a synthetic scheme to make certain compounds according to the invention.

FIG. 5 shows a synthetic schemes to make certain compounds according to the invention.

15 FIG. 6 shows a synthetic schemes to make certain compounds according to the invention.

FIG. 7 shows a synthetic scheme to make a compound according to the invention.

FIG. 8 shows a synthetic scheme to make certain compounds according to the invention.

20 FIG. 9 shows two synthetic schemes to make certain compounds according to the invention.

FIG. 10 shows a synthetic scheme to make certain compounds according to the invention.

FIG. 11 shows the results of *in vivo* testing for weight loss of certain compounds according to the invention.

FIG. 12 shows the results of *in vivo* testing for anti-cancer activity of a compound according to the invention.

5

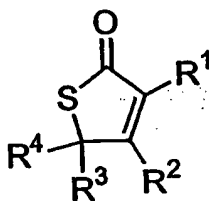
Detailed Description of the Invention

The compounds of the invention can be prepared by conventional means. The synthesis of a number of the compounds is described in the examples. The compounds may be useful for the treatment of obesity, cancer, or microbially-based infections.

10

One embodiment of the invention is compounds having the following general

formula:



I

wherein:

15

$R^1 = H$

$R^2 = -OH, -OR^5, -OCH_2C(O)R^5, -OCH_2C(O)NHR^5, -OC(O)R^5, -OC(O)OR^5, -OC(O)NHNH-R^5,$

or $-OC(O)NR^5R^6$, where R^5 is H, C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, and where R^5 can optionally contain halogen atoms;

R^3 and R^4 , the same or different from each other, are C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl,

20

arylalkyl, or alkylaryl;

with the proviso that when R^2 is $-\text{OH}$, $-\text{OCH}_3$, or $-\text{OC}(\text{O})\text{CF}_3$ and R_3 is $-\text{CH}_3$, then R_4 is not $-\text{CH}_2\text{CH}_2\text{OH}$, $-\text{CH}_2-(\text{C}_6\text{H}_5)$, or $-\text{CH}=\text{CH}-\text{CH}_3$, and
 and the further proviso that when R^3 is $-\text{CH}_2-(\text{C}_6\text{H}_5)$, then R^4 is not $-\text{CH}_3$ or $-\text{CH}_2\text{CH}_3$.

(It should be understood that, when applicable, the keto-tautomeric form of the

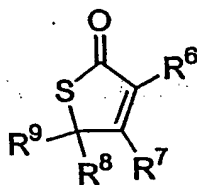
5 foregoing compounds is also included in formula I.)

In a preferred embodiment R^5 is $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

In another preferred embodiment, R^3 is $-\text{H}$ or $-\text{CH}_3$.

In another preferred embodiment, R^4 is $n\text{-C}_6\text{-C}_8$ alkyl.

10 Another embodiment of the invention is compounds formula II



II

wherein

15 $R^6 = \text{C}_2\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, alkynyl, aryl, arylalkyl, or alkylaryl, $-\text{CHR}^{10}\text{OR}^{11}$, $-\text{CO}(\text{O})\text{R}^{10}$, $-\text{C}(\text{O})\text{NR}^{10}\text{R}^{11}$, $-\text{CH}_2\text{C}(\text{O})\text{R}^{10}$, or $-\text{CH}_2\text{C}(\text{O})\text{NHR}^{10}$, where R^{10} and R^{11} are each independently H, $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, but

R^6 is not di-, tri-, or tetra-alkyl substituted phenyl,

$R^7 = -\text{OH}$, $-\text{OR}^{12}$, $-\text{OCH}_2\text{C}(\text{O})\text{R}^{12}$, $-\text{OCH}_2\text{C}(\text{O})\text{NHR}^{12}$, $-\text{OC}(\text{O})\text{R}^{12}$, $-\text{OC}(\text{O})\text{OR}^{12}$,

20 $-\text{OC}(\text{O})\text{NHNH-R}^{12}$, or $-\text{OC}(\text{O})\text{NR}^{12}\text{R}^{13}$, where R^{12} and R^{13} are each independently H, $\text{C}_1\text{-}$

C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, and where R¹² and R¹³ can optionally contain halogen atoms;

R⁸ and R⁹, the same or different from each other, are C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl,

5 with the following provisos:

when R⁶ is ethyl, if R⁸ and R⁹ are not the same, then R⁸ or R⁹ are not ethyl, -CH₂COOH, -

CH₂C(O)NH₂, -CH₂-(C₆H₅), but R⁸ and R⁹ can be the same, even if R⁶ is ethyl,

and

when R⁶ is phenyl, and R⁷ is -OH, R⁸ and R⁹ cannot simultaneously be -CH₃ and

10 -propenyl, and

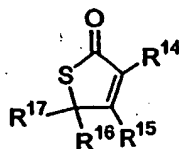
when R⁶ is phenyl, R⁸ and R⁹ cannot simultaneously be -CH₃ or -CH₂-(C₆H₅).

In a preferred embodiment R¹⁰ is C₁-C₁₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

15 In another preferred embodiment, R⁸ is -H or -CH₃.

In another preferred embodiment, R⁹ is n-C₆-C₈ alkyl.

Another embodiment of the invention comprises compounds of formula III:



III

wherein

25 R¹⁴ = -C(O)R¹⁸, where R¹⁸ is H, C₁-C₁₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, optionally containing halogen atoms,

$R^{15} = -OH, -OR^{19}, -OCH_2C(O)R^{19}, -OCH_2C(O)NHR^{19}, -OC(O)R^{19}, -OC(O)OR^{19},$

$-OC(O)NHNH-R^{19},$ or $-OC(O)NR^{19}R^{20},$ where R^{19} and R^{20} are each independently H,

C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, and where R^{19} and R^{20} can

each optionally contain halogen atoms;

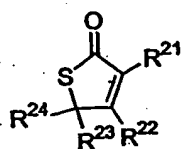
5 R^{16} and R^{17} , the same or different from each other, are C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl,

with the following provisos:

- when R^{14} is $-C(O)CH_3$, and R^{16} and R^{17} are not identical, then either R^{16} or R^{17} are not are not geranyl, p-fluorobenzyl, cinnamyl, farnesyl, methyl, or $-CH_2-(C_6H_5)$, and

10 - when R^{14} is $-C(O)C_6H_5$, then either R^{16} or R^{17} are not are not methyl.

Another embodiment of this invention is a pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I, II, III, or IV:



IV

wherein:

20

$R^{21} = H, C_1-C_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, $-CH_2OR^{25}, -C(O)R^{25},$

$-CO(O)R^{25}, -C(O)NR^{25}R^{26}, -CH_2C(O)R^{25},$ or $-CH_2C(O)NHR^{25},$ where R^{25} and R^{26} are

each independently H, C_1-C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl,

$R^{22} = -OH, -OR^{27}, -OCH_2C(O)R^{27}, -OCH_2C(O)NHR^{27}, -OC(O)R^{27}, -OC(O)OR^{27},$

25 $-OC(O)NHNH-R^{27},$ or $-OC(O)NR^{27}R^{28},$ where R^{27} and R^{28} are each independently H, C_1-

C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, and where R²⁷ and R²⁸ can each optionally contain halogen atoms;

R²³ and R²⁴, the same or different from each other, are C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

- 5 The compositions of the present invention can be presented for administration to humans and other animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, sterile parenteral solutions or suspensions, oral solutions or suspensions, oil in water and water in oil emulsions containing suitable quantities of the compound, suppositories and in fluid suspensions or solutions. As used in this specification, the terms "pharmaceutical diluent"
- 10 and "pharmaceutical carrier," have the same meaning. For oral administration, either solid or fluid unit dosage forms can be prepared. For preparing solid compositions such as tablets, the compound can be mixed with conventional ingredients such as talc, magnesium stearate, dicalcium phosphate, magnesium aluminum silicate, calcium sulfate, starch, lactose, acacia, methylcellulose and functionally similar materials as pharmaceutical diluents or carriers.
- 15 Capsules are prepared by mixing the compound with an inert pharmaceutical diluent and filling the mixture into a hard gelatin capsule of appropriate size. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the compound with an acceptable vegetable oil, light liquid petrolatum or other inert oil.

- Fluid unit dosage forms or oral administration such as syrups, elixirs, and
- 20 suspensions can be prepared. The forms can be dissolved in an aqueous vehicle together with sugar, aromatic flavoring agents and preservatives to form a syrup. Suspensions can be prepared with an aqueous vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

For parenteral administration fluid unit dosage forms can be prepared utilizing the compound and a sterile vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampoule and sealing.

Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. The composition can be frozen after filling into a vial and the water removed under vacuum. The lyophilized powder can then be sealed in the vial and reconstituted prior to use.

The clinical therapeutic indications envisioned for the compounds of the invention include: (1) infections due to invasive micro-organisms such as *staphylococci* and *enterococci*; (2) cancers arising in many tissues whose cells over-express fatty acid synthase, and (3) obesity due to the ingestion of excess calories. Dose and duration of therapy will depend on a variety of factors, including (1) the patient's age, body weight, and organ function (e.g., liver and kidney function); (2) the nature and extent of the disease process to be treated, as well as any existing significant co-morbidity and concomitant medications being taken, and (3) drug-related parameters such as the route of administration, the frequency and duration of dosing necessary to effect a cure, and the therapeutic index of the drug. In general, doses will be chosen to achieve serum levels of 1 ng/ml to 100ng/ml with the goal of attaining effective concentrations at the target site of approximately 1 μ g/ml to 10 μ g/ml.

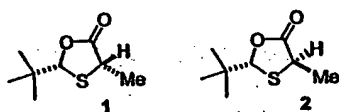
EXAMPLES

The invention will be illustrated, but not limited, by the following examples:

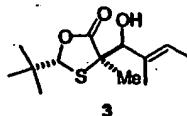
A series of compounds according to the invention were synthesized as described below. Biological activity of certain compounds were profiled as follows: Each compound was tested for: [1] inhibition of purified human FAS, [2] inhibition of fatty acid synthesis activity in

whole cells and [3] cytotoxicity against cultured MCF-7 human breast cancer cells, known to possess high levels of FAS and fatty acid synthesis activity; using the crystal violet and XTT assays. Select compounds with low levels of cytotoxicity were then tested for weight loss in Balb/C mice. In addition, a representative compound from the group which exhibited significant weight loss and low levels of cytotoxicity was tested for its effect on fatty acid oxidation, and carnitine palmitoyltransferase-1 (CPT-1) activity, as well as hypothalamic NPY expression by Northern analysis in Balb/C mice. Certain compounds were also tested for activity against gram positive and/or negative bacteria.

Chemical Synthesis of Compounds

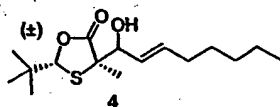


(2*S*,5*R*)-2-*t*-Butyl-5-methyl-1,3-oxathiolan-4-one (1).¹ To a solution of (*S*)-thiolactic acid¹ (4.0 g, 37.7 mmol) in pentane (24 mL) was added trimethylalkynylaldehyde (4.5 mL, 41.5 mmol) and trifluoroacetic acid (TFA) (48 μ L). The solution was heated at reflux using a Dean-Stark Trap for 20 hours. After cooling, the solvent was removed to give a *cis*:*trans* mixture (2.5:1) of 1 and 2 (6.4 g, 99%). Recrystallization (Pentane/Et₂O (8:1) -78 °C) provided pure 1 [α]_D²⁴ = -38 (c 0.4, CHCl₃). ¹H NMR (300 MHz, CDCl₃) *Cis* Isomer δ 0.99 (s, 9 H); 1.53 (d, *J* = 7 Hz, 3 H); 3.94 (q, *J* = 7 Hz, 1 H); 5.17 (s, 1 H). Racemic 1 was also prepared from (\pm)-thiolactic acid.

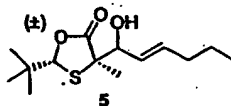


General Procedure A. (2*S*,5*R*)-2-(*t*-Butyl)-5-(1-hydroxy-2-methyl-2-butenyl)-5-methyl-1,3-oxathiolan-4-one (3). To a mixture of diisopropylamine (0.6 mL, 4.6 mmol) in THF (8.0 mL) at -78 °C was added *n*-BuLi (3.3 mL, 1.4 M in *n*-hexane) and the resulting solution was stirred for

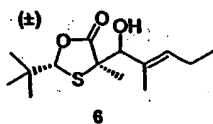
30 minutes at 0 °C and then cooled to -78 °C. Then 1 (800 mg, 4.6 mmol) in THF at -78 °C was added by cannula dropwise and the resulting solution stirred for 30 minutes at -78 °C. Trans 2-methyl-2 butenal (0.4 mL, 4.6 mmol) in THF (1.4 mL), at -78 °C was then added via cannula. After stirring at -78 °C for 1.5 hours, 1 N HCl (25 mL) was added and the solution was extracted with Et₂O (3 x 30 mL). The combined organics were dried (MgSO₄), filtered, and evaporated. Flash chromatography (10% EtOAc/ Hexanes, *r_f* = 0.1) gave 3 (955 mg, 81%) as a 1.6:1 mixture of diastereomers. ¹H NMR (300 MHz, CDCl₃) δ (major diastereomer) 0.99 (s, 9 H), 1.40 (s, 3 H), 1.63 (d, *J* = 6.7 Hz, 3 H), 1.69 (m, 3 H), 4.36 (s, 1 H), 5.25 (s, 1 H), 5.60-5.65 (m, 1 H); (minor diastereomer) 0.98 (s, 9 H), 1.59 (s, 3 H), 1.63 (d, *J* = 6.7 Hz, 3 H), 1.72 (m, 3 H), 4.25 (s, 1 H), 5.07 (s, 1 H), 5.60-5.64 (m, 1 H) ¹³C NMR (75 MHz, CDCl₃) δ (major diastereomer) 12.5, 13.2, 24.3, 24.8, 60.7, 81.8, 87.9, 126.3, 133.8, 178.3; IR (ATR) 3466, 1743 cm⁻¹. Analysis Calculated for C₁₃H₂₂O₃S: C, 60.4; H, 8.58; Found C, 60.4; H, 8.60.



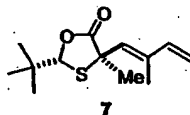
(±)-2-(t-Butyl)-5-(1-hydroxy-2-octenyl)-5-methyl-1,3-oxathiolan-4-one (4). From (±) 1 (800 mg, 4.59) and 2-trans octenal (0.58 mL, 5.1 mmol) following general procedure A was obtained 4 (1.1g, 81%) after flash chromatography (10% EtOAc/Hexanes) as a 1.2:1 mixture of diastereomers. ¹H NMR (300 MHz, CDCl₃) major diastereomer δ 0.85 (t, *J* = 7.2 Hz, 3 H), 0.97 (bs, 9 H), 1.18-1.35 (m, 6 H), 1.56 (s, 3 H), 2.00-2.08 (m, 2 H), 2.38 (d, *J* = 5 Hz, 1 H), 4.15-4.19 (m, 1 H), 5.13 (s, 1 H), 5.45-5.59 (dd, *J* = 7, 14 Hz, 1 H), 5.72-5.77 (m, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.7, 22.3, 24.7, 28.5, 31.3, 32.1, 35.2, 60.6, 78.8, 87.4, 127.2, 136.5, 175.7. ¹H NMR (300 MHz, CDCl₃) minor diastereomer ¹H NMR (300 MHz, CDCl₃) δ 0.85 (t, *J* = 7.2 Hz, 3 H), 0.97 (s, 9 H), 1.18-1.35 (m, 6 H), 1.40 (s, 3 H), 2.00-2.07 (m, 2 H), 2.31 (d, *J* = 5 Hz, 1 H), 4.25-4.30 (m, 1 H), 5.27 (s, 1 H), 5.45-5.59 (dd, *J* = 7, 14 Hz, 1 H), 5.79-5.83 (m, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.7, 22.3, 23.9, 24.8, 28.5, 31.2, 32.1, 35.3, 61.1, 78.3, 87.8, 127.2, 137.2, 177.0. IR (NaCl) 2959, 1765 cm⁻¹. Analysis Calculated for C₁₆H₂₈O₃S: C, 63.9; H, 9.39; Found: C, 63.9; H, 9.41.



(±)-2-(t-Butyl)-5-(1-hydroxy-2-hexenyl)-5-methyl-1,3-oxathiolan-4-one. (5). From (±) 1 (800 mg, 4.59) and 2-trans hexenal (0.58 mL, 5.1 mmol) following general procedure A was obtained 5 (813 mg, 65%) after flash chromatography (10% EtOAc/Hexanes) as a 2.4:1 mixture of diastereomers. ¹H NMR (300 MHz, CDCl₃) δ 0.87 (t, *J* = 7.3 Hz, 3 H), 0.99 (s, 9 H), 1.38-1.45 (m, 2 H), 1.41 (s, 3 H), 2.02 (q, *J* = 7 Hz, 2 H), 4.26-4.31 (m, 1 H), 5.27 (s, 1 H), 5.45-5.63 (m, 1 H), 5.74-5.83 (m, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.6, 21.6, 24.1, 24.9, 35.2, 37.2, 61.2, 78.5, 87.9, 127.3, 137.3, 179.1. IR (NaCl) 2960 1765 cm⁻¹. Analysis Calculated for C₁₄H₂₄O₃S: C, 61.7; H, 8.88; Found: C, 61.74; H, 8.89.

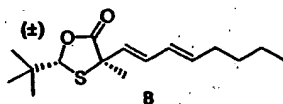


10 (±)-2-(t-Butyl)-5-(1-hydroxy-2-methyl-2-pentenyl)-5-methyl-1,3-oxathiolan-4-one (6). From (±) 1 (800 mg, 4.59 mmol) and 2-methyl-2-pentenal (0.58 mL, 5.0 mmol) following general procedure A was obtained 6 (884 mg, 71%) after flash chromatography (10% EtOAc/Hexanes) as a 1.8:1 mixture of diastereomers. ¹H NMR (300 MHz, CDCl₃) δ 0.93-0.99 (m, 12 H), 1.40 (s, 3 H), 1.68 (s, 3 H), 2.01-2.06 (m 2 H), 4.33 (d, *J* = 6.9 Hz, 1 H), 5.24 (s, 1 H), 5.48-5.54 (m, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 12.6, 13.8, 20.9, 21.1, 24.8, 35.4, 60.6, 81.8, 87.9, 132.6, 133.9, 178.3. IR (NaCl) 2961, 1767 cm⁻¹. Analysis Calculated for C₁₄H₂₄O₃S: C, 61.7; H, 8.88; Found: C, 61.6; H, 8.90.

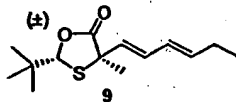


20 General Procedure B. (2S,5R)-2-(t-Butyl)-5-(2-methyl-but-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (7). To a solution of 3 (3.23 g, 12.5 mmol) in Cl(CH₂)₂Cl (115 mL) cooled to 0 °C was added NEt₃ (4.2 mL, 30 mmol) and 2,4- dinitrobenzyl sulfenyl chloride (6.6 g, 28.2 mmol). The solution was warmed to room temperature for 30 minutes or until TLC (10%EtOAc/Hex, *r_f* = .55 major *r_f* = .48 minor) indicated complete formation of the diastereomeric sulfenate esters. The mixture was then refluxed 90 °C for 4 hours or until complete conversion of the sulfenate ester was indicated by TLC. After cooling to 0 °C, pentane (50 mL) was then added and this mixture was filtered through Celite and evaporated. Flash

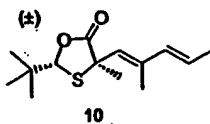
chromatography (2% EtOAc/Hexanes, r_f = 0.4) gave pure 7 (2.3 g, 75%). $[\alpha]_D^{24}$ = +237 (c 1.0, CHCl_3). ^1H NMR (300 MHz, CDCl_3) δ 1.98 (s, 9 H), 1.72 (s, 3 H), 1.86 (s, 3 H), 5.06 (d, J = 10.7 Hz, 1 H), 5.18 (s, 1 H), 5.24 (d, J = 17.3 Hz, 1 H), 5.70 (s, 1 H), 6.24-6.33 (dd, J = 10.7, 17.3 Hz, 1 H); ^{13}C NMR (300 MHz, CDCl_3) δ 12.5, 25.1, 26.6, 34.9, 53.7, 87.4, 113.7, 132.6, 137.8, 140.9, 176.3; Analysis Calculated. for $\text{C}_{13}\text{H}_{20}\text{O}_2\text{S}$: C, 64.9; H, 8.38. Found: C, 63.8; H, 8.28.



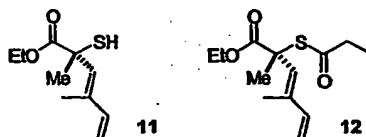
(±)-2-(t-Butyl)-5-(octa-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (8). From (±) 4 (306 mg, 1.00 mmol) following general procedure B was obtained 8 (212 mg, 75%, 4:1 trans:cis) after flash chromatography (2% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) trans isomer δ 0.84-0.89 (m, 3 H), 1.01 (s, 9 H), 1.22-1.38 (m, 4 H), 1.61 (s, 3 H), 2.04-2.11 (m, 2 H), 5.03 (s, 1 H), 5.58 (d, J = 15 Hz, 1 H), 5.64-5.78 (m, 1 H), 0.96-6.05 (m, 1 H), 6.19 (dd, J = 10.1, 15.1 Hz, 1 H). ^{13}C NMR (75 MHz, CDCl_3) trans isomer δ 13.6, 22.0, 22.5, 25.2, 31.2, 32.1, 34.6, 55.9, 87.0, 128.5, 129.6, 130.2, 137.2, 174.7. IR (NaCl) 2959, 1772 cm^{-1} ; HRMS (EI) m/z calculated for $\text{C}_{16}\text{H}_{26}\text{O}_2\text{S}$ (M^+) 282.1653, obsd 282.1681.



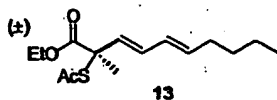
(±)-2-(t-Butyl)-5-(hexa-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (9). From (±) 5 (690 mg, 2.53 mmol) following general procedure B was obtained 9 (461 mg, 72%, 4:1 trans:cis) after flash chromatography (2% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.95-1.01 (m, 12 H), 1.61 (s, 3 H), 2.07-2.12 (m, 2 H), 5.05 (s, 1 H), 5.58 (d, J = 15 Hz, 1 H), 5.81 (dt, J = 6, 15 Hz, 1 H), 6.00-6.05 (m, 1 H), 6.15-6.24 (dd, J = 10, 15.2 Hz, 1 H); ^{13}C (75 MHz, CDCl_3) δ 13.3, 24.8, 25.3, 25.7, 34.5, 56.1, 87.2, 127.4, 129.4, 130.0, 138.9, 175.1. IR (NaCl) 2966, 1771 cm^{-1} . HRMS (ES) m/z calculated for $\text{C}_{14}\text{H}_{22}\text{O}_2\text{SNa}^+$ ($M + \text{Na}^+$) 277.1232, obsd 277.1237.



(±)-2-(*t*-Butyl)-5-(2-methyl-penta-1,3-dienyl)-5-methyl-1,3-oxathiolan-4-one (10). From (±) 6 (500 mg, 2.51 mmol) following general procedure B was obtained 10 (342 mg, 73% 14:1 trans:cis) after flash chromatography (2% EtOAc/Hexanes). ¹H NMR (300 MHz, CDCl₃) δ 1.00 (s, 9 H), 1.70 (s, 3 H), 1.75 (d, *J* = 6.6 Hz, 3 H), 1.85 (s, 3 H), 5.18 (s, 1 H), 5.57 (s, 1 H), 5.75 (dq, *J* = 6.6, 16 Hz, 1 H), 5.97 (d, *J* = 16 Hz, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.0, 18.0, 25.2, 27.4, 34.8, 53.8, 87.4, 125.4, 129.3, 135.5, 137.8, 176.3. IR (NaCl) 2961, 1770 cm⁻¹. HRMS (EI) *m/z* calculated for C₁₄H₂₂O₂S (M⁺) 254.1341, obsd 254.1309.

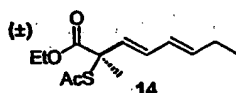


General Procedure C. 2-(*R*)-2,4-Dimethyl-2-thiopropionyl-hexa-3,5-dienoic acid ethyl ester (12). Cesium carbonate (332 mg, 1.0 mmol) was added directly to a solution of 7 (250 mg, 1.0 mmol) in EtOH (3.9 mL). After 20 minutes this mixture was poured into a mixture of NH₄Cl(sat)/1 N HCl (15 mL, 3:1) and extracted with Et₂O (3 x 20 mL). The combined organics were dried (MgSO₄), filtered and evaporated to give crude 11. To 11 was added CH₂Cl₂ (7.5 mL) and the solution was cooled to 0 °C. NEt₃ (0.14 mL, 1.0 mmol) and propionyl chloride (.09 mL, 1.0 mmol) were added and the solution stirred at 0 °C. After 40 minutes. NH₄Cl (sat) (20 mL) was added and this mixture was extracted with CH₂Cl₂ (3 x 15 mL). The combined organics were dried (MgSO₄), filtered and evaporated. Flash chromatography (5% EtOAc/Hex, *rf* = 0.4) gave pure 12 (261 mg, 72%). [α]_D²³ = + 4.2 (*c* 0.9, CHCl₃) ¹H NMR (300 MHz, CDCl₃) δ 1.11 (t, *J* = 7.4 Hz, 3 H), 1.23 (t, *J* = 7.0 Hz, 3 H), 1.83 (s, 3 H), 1.85 (s, 3 H), 2.48 (q, *J* = 7.5 Hz, 2 H), 4.18 (q, *J* = 6.9 Hz, 2 H), 5.02 (d, *J* = 10.7 Hz, 1 H), 5.18 (d, *J* = 17.3 Hz, 1 H), 5.73 (s, 1 H), 6.24-6.34 (dd, *J* = 10.7, 17.3 Hz, 1 H); ¹³C NMR (300 MHz, CDCl₃) δ 9.43, 12.9, 13.9, 26.1, 36.5, 55.2, 61.9, 113.1, 131.4, 138.2, 141.4, 172.1, 198.9. IR (NaCl) 2981, 1735, 1694 cm⁻¹. HRMS (EI) *m/z* calculated for C₁₃H₂₀O₃S (M⁺) 256.1133 obsd 256.1127.

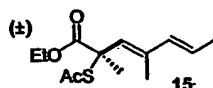


(±)- 2-Thioalkynyl-2-methyl-deca-3,5-dienoic acid ethyl ester (13). From 8 (200 mg, 0.71 mmol) and alkynyl chloride (55 μL, 0.78 mmol) following general procedure C gave 13 (119 g

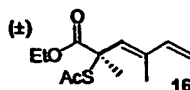
- 59%) after flash chromatography (5% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.84-0.89 (m, 3 H), 1.23 (t, $J=7.1$ Hz, 3 H), 1.28-1.38 (m, 4 H), 1.71 (s, 3 H), 2.01-2.08 (m, 2 H), 2.23 (s, 3 H), 4.18 (q, $J=7.1$ Hz, 2 H), 5.66-5.76 (m, 2 H), 5.89-6.03 (m, 1 H), 6.20 (dd, $J=10.3, 15.3$ Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.8, 13.9, 22.2, 22.8, 29.9, 31.2, 32.3, 56.1, 61.9, 128.4, 129.2, 132.2, 137.1, 171.6, 194.6. IR (NaCl) 2930, 1737, 1694 cm^{-1} . HRMS (ES) m/z calculated for $\text{C}_{15}\text{H}_{24}\text{O}_3\text{SNa}^+$ ($M+\text{Na}^+$) 307.1338 obsd. 307.1339.



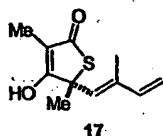
- (±)- 2-Thioalkynyl-2-methyl-octa-3,5-dienoic acid ethyl ester (14). From 9 (353 mg, 1.39 mmol) and alkynyl chloride (98 mL, 1.39mmol) following general procedure C gave 14 (142 g, 40%) after flash chromatography (5% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.83 (t, $J=7.3$ Hz, 3 H), 1.24 (t, $J=7.1$ Hz, 3 H), 1.72 (s, 3 H), 2.03-2.17 (m, 2 H), 2.25 (s, 3 H), 4.17 (q, $J=7.1$ Hz, 2 H), 5.72-5.81 (m, 2 H), 5.95-6.04 (dd, $J=10, 15$ Hz, 1 H), 6.18-6.27 (dd, $J=10, 15$ Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.2, 13.9, 22.8, 25.6, 30.2, 56.1, 61.9, 128.2, 128.4, 132.1, 138.5, 171.6, 194.8. IR (NaCl) 2929, 1736, 1693 cm^{-1} ; HRMS (ES) m/z calculated for $\text{C}_{13}\text{H}_{20}\text{O}_3\text{SNa}^+$ ($M+\text{Na}^+$) 279.1025 obsd 279.1032.



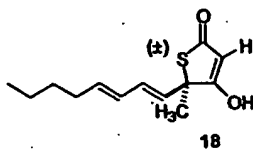
- (±)- 2-Thioalkynyl-2,4-dimethyl-hepta-3,5-dienoic acid ethyl ester (15). From 10 (369 mg, 1.46 mmol) and alkynyl chloride (103 μL , 1.46mmol) following general procedure C gave 15 (271 mg, 77 %) after flash chromatography (5% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 1.26 (t, $J=7.1$ Hz, 3 H), 1.74 (d, $J=6.6$ Hz, 3 H), 1.81 (s, 3 H), 1.85 (s, 3 H), 2.25 (s, 3 H), 4.17 (q, $J=7.1$ Hz, 2 H), 5.56 (s, 1 H), 5.65-5.73 (dq, $J=6.6, 16$ Hz, 1 H), 5.99 (d, $J=16$ Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.8, 14.1, 18.2, 26.2, 30.5, 55.6, 62.0, 125.2, 128.3, 135.7, 138.5, 172.2, 194.8. IR (NaCl) 2926, 1737, 1694 cm^{-1} ; HRMS (EI) m/z calculated for $\text{C}_{13}\text{H}_{20}\text{O}_3\text{S}$ (M^+) 256.1133 obsd 256.1118.



(±)- 2-Thioalkynyl-2,4-dimethyl-hexa-3,5-dienoic acid ethyl ester (16). From (±) 7 (380 mg, 1.56 mmol) and alkynyl chloride (110 μ L, 1.56 mmol) following general procedure C gave 16 (230 mg, 61%) after flash chromatography (5% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 1.25 (t, $J = 7.1$ Hz, 3 H), 1.84 (s, 3 H), 1.87 (s, 3 H), 2.24 (s, 3 H), 4.21 (q, $J = 7.1$ Hz, 2 H), 5.03 (d, $J = 10.6$ Hz, 1 H), 5.21 (d, $J = 17.3$ Hz, 1 H), 5.74 (s, 1 H), 6.26-6.35 (dd, $J = 10.6$, 17.3 Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 12.9, 13.9, 25.9, 30.1, 55.8, 62.0, 113.3, 131.3, 138.3, 141.3, 182.3, 194.6. IR (NaCl) 2982, 1735, 1692 cm^{-1} .

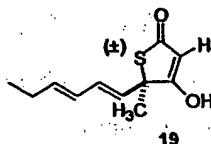


General Procedure D. 5-(*R*)-4-Hydroxy-3,5-dimethyl-5-(2-methyl-buta-1,3-dienyl)-5-*H*-thiophen-2-one (17) (Thiolactamycin). To 12 (315 mg, 1.23 mmol) in THF (18.5 mL) at -78°C was added LiHMDS (3.1 mL, 3.1 mmol, 1.0 M in THF) and the solution was allowed to slowly warm to -5°C . The solution was then poured into 1 N HCl (25 mL) and extracted with EtOAc (3 x 15 mL). The combined organics were dried (MgSO_4), filtered and evaporated. This crude mixture was taken up in NaHCO_3 (sat, 15 mL) and extracted with Et_2O (3 x 10 mL). The aqueous layer was then acidified to pH 3 (pH paper) with 1 N HCl and extracted with Et_2O (3 x 10 mL) and EtOAc (2 x 10 mL). The combined organics were dried (MgSO_4), filtered and evaporated to provide pure 17. (182 mg, 70%, 96% ee). Recrystallization from Hexanes/Acetone (3:1) gave optically enriched 17. $[\alpha]_D^{24} = +174$ (c 0.6, MeOH), mp $119.5\text{--}121^\circ\text{C}$ (lit $[\alpha]_D^{20} +176$ (c 1.0, MeOH), mp 120°C)². ^1H NMR (300 MHz, CDCl_3) δ 1.72 (s, 3 H), 1.76 (s, 3 H), 1.91 (s, 3 H), 5.05 (d, $J = 10.7$ Hz, 1 H), 5.23 (d, $J = 17.3$ Hz, 1 H), 5.58 (s, 1 H), 6.23-6.33 (dd, $J = 10.7$, 17.3 Hz, 1 H); ^{13}C NMR (300 MHz, CDCl_3) δ 7.60, 12.0, 29.8, 55.3, 110.6, 113.9, 129.1, 140.3, 140.7, 179.2, 196.7. IR (NaCl) 3422, 1607 cm^{-1} . Analysis Calculated for $\text{C}_{11}\text{H}_{14}\text{O}_2\text{S}$: C, 62.8; H, 6.71; Found: C, 62.1, 6.71.

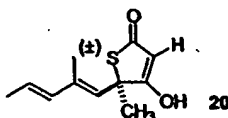


(±)-4-Hydroxy-5-methyl-5-octa-1,3-dienyl-5-*H*-thiophen-2-one (18). From 13 (62 mg, 0.22 mmol) following general procedure D was obtained 18 (21 mg, 41%). ^1H NMR (300 MHz,

CDCl₃) (keto tautomer) δ 0.88 (t, J = 6.9 Hz, 3 H), 1.19-1.41 (m, 4 H), 1.75 (s, 3 H), 2.03-2.19 (m, 2 H), 3.22 (d, J = 21 Hz, 1 H), 3.51 (d, J = 21 Hz, 1 H), 5.67 (d, J = 15 Hz, 1 H), 5.80 (dt, J = 7, 17 Hz, 1 H), 6.02 (dd, J = 10, 15 Hz, 1 H), 6.37 (dd, J = 10, 15 Hz, 1 H). ¹H NMR (300 MHz, MeOD) enol tautomer δ 0.97-1.03 (m, 3 H), 1.36-1.53 (m, 4 H), 1.87 (s, 3 H), 2.15-2.22 (m, 2 H), 5.78 (d, J = 15 Hz, 1 H), 5.82- 5.90 (m, 1 H), 6.10-6.19 (m, 1 H), 6.38 (dd, J = 10.3, 15.4 Hz, 1 H); ¹³C (75 MHz, MeOD) enol tautomer δ 14.4, 23.3, 25.2, 32.6, 33.4, 60.9, 102.1 (m), 130.7, 131.7, 132.7, 137.5, 188.9, 196.9. IR (NaCl) 2927, 1588 cm⁻¹; HRMS (ES) calculated for C₁₃H₁₈O₂SN⁺ (M+Na⁺) 261.0911; obsd 261.0912.

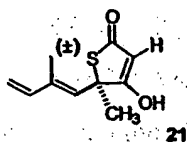


10 (±)-4-Hydroxy-5-methyl-5-hexa-1,3-dienyl-5-*H*-thiophen-2-one (19). From 14 (364 mg, 0.46 mmol) following general procedure D was obtained 19 (180 mg, 60 %). ¹H (300 MHz, CDCl₃, exists as a mixture 2.3:1 of the keto:enol tautomer) keto tautomer: δ 1.00 (t, J = 7.4 Hz, 3 H); 1.76 (s, 3 H); 2.09-2.16 (m, 2 H); 3.21 (d, J = 21 Hz, 1 H); 3.52 (d, J = 21 Hz, 1 H); 5.70 (d, J = 15 Hz, 1 H); 5.86 (dt, J = 15 Hz, 6 Hz, 1 H), 6.02 (dd, J = 10, 15 Hz, 1 H), 6.38 (dd, J = 15, 10 Hz, 1 H); ¹H NMR (300 MHz, MeOD) enol tautomer δ 1.09 (t, J = 7.4 Hz, 3 H), 1.87 (s, 3 H), 2.14-2.29 (m, 2 H), 5.78 (d, J = 15 Hz, 1 H), 5.87 (dt, J = 15, 6.57 Hz, 1 H), 6.09-6.18 (m, 1 H), 6.38 (dd, J = 10.2, 15 Hz, 1 H); ¹³C NMR (75 MHz, MeOD) enol tautomer δ 14.1, 25.2, 26.9, 61.0, 101 (m), 129.7, 131.7, 132.7, 138.9, 188.9, 197.1. IR (NaCl) 2965, 1592 cm⁻¹; HRMS (ES) m/z calculated for C₁₁H₁₄O₂SN⁺ (M+Na⁺) 233.0607, obsd 233.0626.

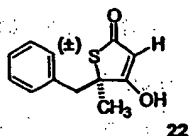


20 (±)-4-Hydroxy-5-methyl-5-(2-methyl-penta-1,3-dienyl)-5-*H*-thiophen-2-one (20). From 15 (226 mg, 0.9 mmol) following general procedure D was obtained 20 (95 mg, 49%). ¹H NMR (300 MHz, CDCl₃) (keto-tautomer) δ 1.75 (s, 3 H), 1.77 (d, J = 3.2 Hz, 3 H), 1.84 (s, 3 H), 3.42 (d, J = 1.5 Hz, 2 H), 5.43 (d, J = 21 Hz, 1 H), 5.66 (bs, 1 H), 5.78 (dd, J = 6, 22 Hz, 1 H), 6.04 (d, J = 15 Hz, 1 H); ¹H NMR (300 MHz, MeOD) (enol tautomer) δ 1.80-1.85 (m, 6 H), 1.90 (s, 3 H), 5.59 (s, 1 H), 5.80-5.95 (m, 1 H), 6.17 (d, J = 15 Hz, 1 H); ¹³C NMR (75 MHz, MeOD) (enol

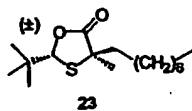
tautomer) δ 13.4, 18.4, 30.7, 59.2, 101.2 (m) 126.2, 128.4, 136.9, 140.6, 190.2, 197.6. IR (NaCl) 2929, 1593 cm^{-1} ; HRMS (ES) m/z calculated for $\text{C}_{11}\text{H}_{14}\text{O}_2\text{SNa}^+$ ($\text{M}+\text{Na}^+$) 233.0607 obsd. 233.0597.



5. **(±)-4-Hydroxy-5-methyl-5-(2-methyl-but-1,3-dienyl)-5-H-thiophen-2-one (21).** From 16 (181 mg, 0.75 mmol) following general procedure D was obtained 21 (66 mg, 45%). ^1H NMR (300 MHz, CDCl_3) (keto tautomer) δ 1.78 (s, 3 H), 1.86 (s, 3 H), 3.43 (d, $J = 5.6$ Hz, 2 H), 5.12 (d, $J = 10.6$ Hz, 1 H), 5.27 (d, $J = 17.3$ Hz, 1 H), 5.83 (s, 1 H), 6.27-6.37 (dd, $J = 10.6, 17.3$ Hz, 1 H). ^1H NMR (300 MHz, MeOD) (enol tautomer) δ 1.79 (s, 3 H), 1.84 (s, 3 H), 5.04 (d, $J = 10.7$ Hz, 1 H), 5.25 (d, $J = 17.3$ Hz, 1 H), 5.66 (s, 1 H), 6.36 (dd, $J = 10.7, 17.3$ Hz, 1 H); ^{13}C NMR (75 MHz, MeOD) δ 12.6, 30.4, 59.0, 102 (m), 116.9, 131.4, 140.6, 142.3, 189.9, 197.3. HRMS (EI) m/z calculated for $\text{C}_{10}\text{H}_{12}\text{O}_2\text{S}^+$ (M^+) 196.0552 obsd. 196.0552.



15. **(±)-5-Benzyl-4-hydroxy-5-methyl-5-H-thiophen-2-one (22).** From 31 (1.4 mg, 5.0 mmol) following general procedure D was obtained 22 (500 mg, 45 %). ^1H NMR (300 MHz, CDCl_3) δ 1.71 (s, 3 H), 2.89 (ab q, $J = 22$ Hz, 2 H), 3.17 (ab q, $J = 14$ Hz, 2 H), 7.26 (m, 5 H); ^{13}C NMR (75 MHz, CDCl_3) δ 26.2, 46.6, 48.5, 67.9, 127.7, 128.6, 130.6, 134.9, 195.3, 207.3.

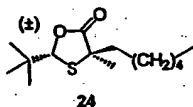


20

General Procedure E. (±)-2-tert-butyl-5-methyl-5-octyl-[1,3]-oxathiolan-4-one (23). To a mixture of LiHMDS (6.2 mL, 6.20 mmol, 1 M in THF) in THF (9.7 mL) at -78°C was added (±)-1 (1.00 g, 5.75 mmol) in THF (9.60 mL) by cannula dropwise, and the resulting solution stirred for 30 minutes. at -78°C . Then, octyl triflate (1.63 g, 6.20 mmol) in THF (4 mL) at -78°C .

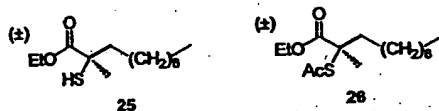
°C was added via cannula. After stirring at -78 °C for 2 hours, 1 N HCl (10 mL) was added and the solution was extracted with Et₂O (3 x 15 mL). The combined organics were dried (MgSO₄), filtered and evaporated. Flash chromatography (2% EtOAc/Hexanes) gave pure 23 as a 2:1-6:1 mixture of separable diastereomers (1.33 g, 81%). ¹H NMR (300 MHz, CDCl₃) δ 0.86 (t, J= 6.5 Hz, 3 H), 0.99 (s, 9 H), 1.24-1.26 (m, 12 H), 1.54 (s, 3 H), 1.72-1.84 (m, 2 H), 5.13 (s, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.9, 22.6, 24.9, 25.1, 25.9, 29.2, 29.3, 29.5, 31.8, 35.2, 41.2, 55.3, 86.5, 177.7. IR (NaCl) 3443, 2929, 1829, 1769 cm⁻¹; Analysis Calculated. for C₁₆H₃₀O₂S: C, 67.0; H, 10.6; Found: C, 66.3; H, 10.5. HRMS (EI) m/z calculated for C₁₆H₃₀O₂S⁺ (M⁺) 286.1967 obsd. 286.1969.

10



(±)-2-tert-butyl-5-methyl-5-hexyl-[1,3]-oxathiolan-4-one (24). From (±)-1 (500 mg, 2.87 mL) and hexyl triflate (738 mg, 2.87 mmol) following general procedure E was obtained 24 (557 mg, 75%) as a 2:1-6:1 mixture of separable diastereomers. ¹H NMR (300 MHz, CDCl₃) δ 0.87 (t, J= 6.5 Hz, 3 H), 0.99 (s, 9 H), 1.24-1.29 (m, 8 H), 1.54 (s, 3 H), 1.72-1.80 (m, 2 H), 5.13 (s, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 13.9, 22.5, 24.9, 24.9, 25.1, 25.9, 29.1, 31.6, 41.2, 55.3, 86.7, 177.8. IR (NaCl) Analysis Calculated. for C₁₄H₂₆O₂S: C, 65.1; H, 10.1; Found: C, 64.5; H, 10.1. HRMS (EI) m/z calculated for C₁₄H₂₆O₂S⁺ (M⁺) 258.1654 obsd. 286.1653..

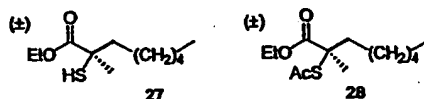
20



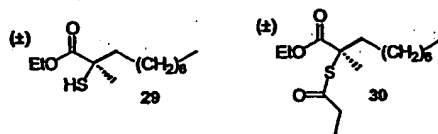
25

General Procedure F. (±)-2-Alkynylsulfanyl-2-methyl-decanoic acid ethyl ester (26). To 23 (650 mg, 2.27 mmol) in EtOH (14.1 mL) was added NaOEt (2.1 M) (2.16 mL, 4.54 mmol) (freshly prepared from Na metal (200 mg, 8.3 mmol) in EtOH (4.0 mL)) and the solution was allowed to stir at room temperature. After 2 hours, the solution was poured into NH₄Cl (sat)/1 N HCl (25 mL, 3:1) and this mixture was extracted with Et₂O (3 x 20 mL). The combined organics were then washed with H₂O (3 x 25 mL), dried (MgSO₄), filtered and evaporated to give crude 25. To 25 dissolved in CH₂Cl₂ (26 mL) at 0 °C was added NEt₃ (0.5 mL, 3.49 mmol) and

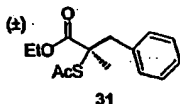
alkynyl chloride (0.3 mL, 3.49 mmol). After 40 minutes at 0 °C, $\text{NH}_4\text{Cl}_{(\text{sat})}$ (30 mL) was added and the solution was extracted with CH_2Cl_2 . The combined organics were dried (MgSO_4), filtered and evaporated. Flash chromatography (5% EtOAc/Hexanes) gave pure 26 (542 mg, 79%). ^1H NMR (300 MHz, CDCl_3) δ 0.87 (t, $J = 6.9$ Hz, 3 H), 1.22-1.27 (m, 15 H), 1.61 (s, 3 H), 1.75-1.84 (m, 2 H), 2.26 (s, 3 H), 4.18 (q, $J = 7.1$ Hz, 2 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.9, 14.1, 22.6, 23.4, 24.4, 29.1, 29.2, 29.6, 30.3, 31.8, 38.3, 55.8, 61.5, 173.1, 195.8. IR (NaCl) 3430, 1868, 1693, 1644 cm^{-1} ; Analysis Calculated. for $\text{C}_{15}\text{H}_{28}\text{O}_3\text{S}$: C, 62.5; H, 9.78; Found: C, 62.6; H, 9.83.



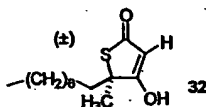
- 10 (±)-2-Alkynylsulfanyl-2-methyl-octanoic acid ethyl ester (28) From 24 (940 mg, 3.63 mmol) and alkynyl chloride (0.3 mL, 3.63 mmol) following general procedure F was obtained 28 (727mg, 77%) after flash chromatography (5% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.9$ Hz, 3 H), 1.22-1.27 (m, 11 H), 1.61 (s, 3 H), 1.75-1.79 (m, 2 H), 2.25 (s, 3 H), 4.17 (q, $J = 7$ Hz, 2 H); ^{13}C NMR (75 MHz, CDCl_3) δ 13.9, 14.1, 22.4, 23.4, 24.4, 29.3, 30.3, 31.5, 38.4, 55.7, 61.5 173.0, 194.7. IR (NaCl) 3449, 1736, 1694 cm^{-1} ; Analysis Calculated. for $\text{C}_{13}\text{H}_{24}\text{O}_3\text{S}$: C, 59.9; H, 9.29; Found: C, 60.6; H, 9.44.



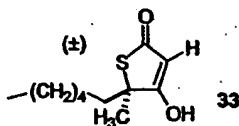
- 20 (±)-2-Methyl 2-propionylsulfanyl-decanoic acid ethyl ester (30). From 23 (613 mg, 2.14 mmol) and propionyl chloride (0.19 mL, 2.14 mmol) following general procedure F was obtained 30 (484 mg, 75%) after flash chromatography (5% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.84 (t, $J = 6.9$ Hz, 3 H), 1.10 (t, $J = 7.5$ Hz, 3 H), 1.19-1.24 (m, 15 H), 1.58 (s, 3 H), 1.72-1.77 (m, 2 H), 2.48 (q, $J = 7.5$ Hz, 2 H), 4.17 (q, $J = 7$ Hz, 2 H); ^{13}C NMR (75 MHz, CDCl_3) δ 9.45, 14.1, 14.1, 22.6, 23.5, 24.5, 29.1, 29.3, 29.7, 31.8, 36.9, 38.5, 55.5, 61.4, 173.2, 199.2. Analysis Calculated for $\text{C}_{16}\text{H}_{30}\text{O}_3\text{S}$: C, 63.5; H, 10.0; Found: C, 63.7; H, 10.0.



(±)-2-Alkynylsulfanyl-2-methyl-3-phenyl-decanoic acid ethyl ester (31). From 5-Benzyl-2-tert-butyl-5-methyl-[1,3]oxathiolan-4-one¹. (1.2 g, 4.7 mmol) following general procedure F was obtained 31 (954 mg, 76%) after flash chromatography (5% EtOAc/Hexanes). ¹H NMR (300 MHz, CDCl₃) δ 1.19 (t, *J* = 7 Hz, 3 H), 1.55 (s, 3 H), 2.26 (s, 3 H), 3.13 (q, *J* = 13 Hz, 2 H), 4.13 (q, *J* = 7 Hz, 2 H), 7.1 (m, 2 H), 7.2 (m, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 14.0, 23.1, 30.3, 43.6, 56.3, 61.7, 127.2, 128.1, 130.7, 135.4, 172.8, 194.8.

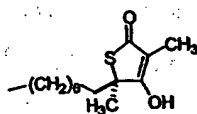


General Procedure G. (±)- 4-Hydroxy-5-methyl-5-octyl-5-H-thiophen-2-one (32). To 26 (500 mg, 1.7 mmol) in toluene (27 mL) at -78 °C was added LiHMDS (4.3 mL, 4.3 mmol, 1.0 M in THF) and the solution was allowed to slowly warm to -5 °C. The solution was then poured into 1 N HCl (40 mL) and extracted with EtOAc (3 x 25 mL). The combined organics were dried (MgSO₄), filtered and evaporated. Flash chromatography (20% EtOAc/2% CH₃CO₂H/ Hexanes) gave 32 (308 mg, 73%). ¹H NMR (300 MHz, CDCl₃) (keto-tautomer) δ 0.86 (t, *J* = 6 Hz, 3 H), 1.19-1.24 (m, 10 H), 1.48-1.53 (m, 2 H), 1.65 (s, 3 H), 1.77-1.85 (m, 1 H), 1.94-2.01 (m, 1 H), 3.36 (s, 2 H); ¹H NMR (300 MHz, MeOD) (enol tautomer) 0.87-0.89 (m, 3 H), 1.29 (m, 10 H), 3.29 (s, 3 H), 1.81-1.87 (m, 2 H); ¹³C NMR (75 MHz, MeOD) (enol tautomer) δ 14.7, 23.8, 26.4, 27.1, 30.5, 30.6, 30.8, 33.2, 39.8, 61.3, 103.1 (m), 189.8, 197.8. IR (NaCl) 3422, 1593 cm⁻¹; Analysis Calculated for C₁₃H₂₂O₂S: C, 64.4; H, 9.15; Found: C, 64.3; H, 9.10.



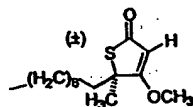
(±)- 4-Hydroxy-5-methyl-5-hexyl-5-H-thiophen-2-one (33). From 28 (715 mg, 2.75 mmol) following general procedure G was obtained 33 (402 mg, 69%) after flash chromatography (20% EtOAc/2% CH₃CO₂H/Hexanes). ¹H NMR (300 MHz, CDCl₃) δ (keto tautomer) 0.86 (t, *J* = 7 Hz, 3 H), 1.27 (bs, 8 H), 1.68 (s, 3 H), 1.94-2.26 (m, 2 H), 3.35 (s, 2 H). ¹H NMR (300 MHz, MeOD) (enol tautomer) δ 0.89 (t, *J* = 6.5 Hz, 3 H), 1.21-1.36 (m, 7 H), 1.46-1.54 (m, 1 H), 1.64

(s, 3 H), 1.80-1.90 (m, 2 H); ^{13}C NMR (75 MHz, MeOD) δ 14.6, 23.8, 26.3, 27.1, 30.5, 32.9, 39.8, 61.3, 103.5 (m), 189.8, 197.8. Analysis Calculated for $\text{C}_{11}\text{H}_{18}\text{O}_2\text{S}$: C, 61.6; H, 8.47; Found: C, 61.7; H, 8.67.



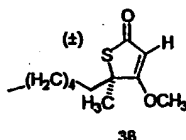
34

5 (±)-4-Hydroxy-3,5-dimethyl-5-octyl-5-H-thiophen-2-one (34). From 30 (469 mg, 1.55 mmol) and NaHMDS (3.87 mL, 3.87 mmol, 1.0 M in THF) following general procedure G was obtained 34 (397 mg, 70%). ^1H NMR (300 MHz, CDCl_3) (enol tautomer) δ 0.86 (t, $J = 6.8$ Hz, 3 H), 1.23 (s, 11 H), 1.30-1.45 (m, 1 H), 1.59 (s, 3 H), 1.74 (s, 3 H), 1.84-1.88 (m, 2 H); ^{13}C NMR (75
10 MHz, CDCl_3) δ 7.48, 14.0, 22.6, 25.2, 25.9, 29.2, 29.4, 29.6, 31.8, 38.5, 58.2, 110.5, 180.9, 198.0. IR (NaCl) 2927, 1601 cm^{-1}

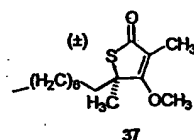


35

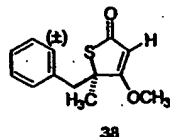
General Procedure H. (±)-4-Methoxy-5-methyl-5-octyl-5-H-thiophen-2-one (35). To 32 (70 mg, 0.27 mmol) in DMF (1.1 mL) cooled to -40°C was added NaH (14 mg, 0.35 mmol, 60% in
15 mineral oil) and the solution was allowed to warm and stir at 0°C for 30 minutes. Dimethyl sulfate (50 μL , 0.55 mmol) was then added directly and the mixture was allowed to warm and stir for 2.5 hours at room temperature. $\text{NH}_4\text{Cl}_{(\text{sat})}/1\text{ N HCl}$ (3:1, 10 mL) was added and the solution was extracted with Et_2O (3 x 10 mL). The combined organics were washed with H_2O (3 x 15 mL), dried (MgSO_4), filtered and evaporated. Flash chromatography (15% EtOAc/Hexanes)
20 gave pure 35 (59 mg, 80%). ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t, $J = 7$ Hz, 3 H); 1.07-1.18 (m, 1 H), 1.23 (s, 10 H), 1.43-1.49 (m, 1 H), 1.61 (s, 3 H), 1.74-1.81 (m, 2 H), 3.81 (s, 3 H), 5.29 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 22.6, 25.1, 26.4, 29.1, 29.3, 29.5, 31.8, 38.8, 59.3, 59.4, 101.3, 187.3, 193.8. Analysis. Calculated for $\text{C}_{14}\text{H}_{24}\text{O}_2\text{S}$: C, 65.6; H, 9.50; Found: C, 65.8; H, 9.50.



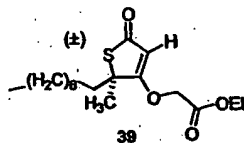
(±)- 4-Methoxy-5-methyl-5-hexyl-5-*H*-thiophen-2-one (36). From 33 (40.3 mg, 0.19 mmol) and dimethyl sulfate (35 μ L, 0.37 mmol) following general procedure H was obtained 36 (25 mg, 58%) after flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.7$ Hz, 3 H), 1.08-1.13 (m, 1 H), 1.24 (s, 6 H), 1.35-1.39 (m, 1 H), 1.61 (s, 3 H), 1.75-1.82 (m, 2 H), 3.81 (s, 3 H), 5.30 (s, 1 H); ^{13}C NMR (75 Mz, CDCl_3) δ 14.0, 22.5, 25.1, 26.4, 29.2, 31.5, 38.9, 59.4, 59.4, 101.3, 187.3, 193.8.



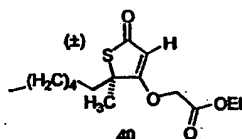
(±)- 4-Methoxy-3,5-dimethyl-5-octyl-5-*H*-thiophen-2-one (37). From 34 (40 mg, 0.16 mmol), KH (27 mg, 0.20 mmol, 30% in mineral oil) and dimethyl sulfate (30 μ L, 0.31 mmol) following general procedure H was obtained 37 (30 mg, 71%). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.06-1.09 (m, 1 H), 1.24 (s, 10 H), 1.41-1.48 (m, 1 H), 1.55 (s, 3 H), 1.71-1.79 (m, 2 H), 1.98 (s, 3 H), 4.09 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 9.59, 14.1, 22.6, 25.2, 26.5, 29.2, 29.4, 29.6, 31.8, 38.9, 58.7, 59.8, 111.3, 180.2, 195.7. IR (NaCl) 2927, 1676, 1631, 1582 cm^{-1} . Analysis Calculated for $\text{C}_{15}\text{H}_{26}\text{O}_2\text{S}$: C, 66.6; H, 9.69; Found: C, 66.5; H, 9.67.



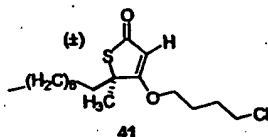
(±)-5-Benzyl-4-methoxy-5-methyl-5-*H*-thiophen-2-one (38). From 22 (50 mg, 0.23 mmol), and dimethyl sulfate (44 μ L, 0.45 mmol) following general procedure H was obtained 38 (38 mg, 74 %). ^1H NMR (300 MHz, CDCl_3) δ 1.65 (s, 3 H), 3.1 (q, $J = 7$ Hz, 2 H), 3.84 (s, 3 H), 5.19 (s, 1 H), 7.21 (m, 5 H); ^{13}C NMR (75 MHz, CDCl_3) δ 26.0, 45.0, 59.3, 59.9, 101.9, 127.2, 128.0, 130.4, 135.9, 186.5, 192.9.



(±)-5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetic acid ethyl ester (39). From 32 (39 mg, 0.16 mmol) and ethyl bromoacetate (36 μ L, 0.32 mmol) following general procedure H was obtained 39 (39 mg, 73%) after flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, J = 6 Hz, 3 H), 1.24 (s, 11 H), 1.29 (t, J = 7 Hz, 3 H), 1.47-1.48 (m, 1 H), 1.68 (s, 3 H), 1.85-1.88 (m, 2 H), 4.25 (q, J = 7 Hz, 2 H), 4.54 (s, 2 H), 5.20 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 14.1, 22.6, 25.1, 26.4, 29.2, 29.3, 29.5, 31.8, 38.8, 59.7, 61.9, 67.9, 102.3, 166.2, 185.3, 193.4. IR (NaCl) 2928, 1762, 1682, 1612 cm^{-1} . Analysis Calculated for $\text{C}_{17}\text{H}_{28}\text{O}_4\text{S}$: C, 62.2; H, 8.59; Found: C, 62.2; H, 8.67.

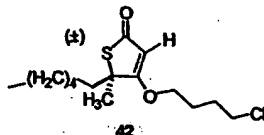


(±)-5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)-acetic acid ethyl ester (40). From 33 (20 mg, 0.09 mmol) and ethyl bromoacetate (20 μ L, 0.2 mmol) following general procedure H was obtained 40 (18 mg, 67%) after flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, J = 6.8 Hz, 3 H), 1.24-1.27 (m, 7 H), 1.32 (t, J = 7 Hz, 3 H), 1.47-1.48 (m, 1 H), 1.68 (s, 3 H), 1.84-1.88 (m, 2 H); 4.25 (q, J = 7 Hz, 2 H), 4.54 (s, 2 H), 5.21 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 14.1, 22.5, 25.1, 26.4, 29.2, 31.6, 38.9, 59.7, 61.9, 68.0, 102.3, 166.2, 185.3, 193.3. IR (NaCl) 2932, 1762, 1682, 1612 cm^{-1} . Analysis Calculated for $\text{C}_{15}\text{H}_{24}\text{O}_4\text{S}$: C, 59.9; H, 8.05; Found: C, 59.9; H, 8.08.



(±)-4-(4-Chloro-butoxy)-5-methyl-5-octyl-5H-thiophen-2-one (41). From 32 (47 mg, 0.18 mmol) and 3-iodo-1-chlorobutane (40 μ L, 0.36 mmol) following general procedure H was obtained 41 (46 mg, 85%) after flash chromatography (20% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, J = 7 Hz, 3 H), 1.07-1.27 (m, 1 H), 1.24 (s, 10 H), 1.48-1.51 (m, 1 H),

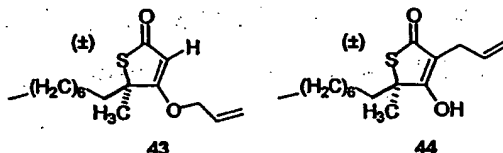
1.62 (s, 3 H), 1.75-1.82 (m, 2 H), 1.89-1.98 (m, 4 H), 3.59 (t, $J = 5.9$ Hz, 2 H), 3.95-3.98 (m, 2 H), 5.28 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 22.6, 25.1, 26.0, 26.5, 29.0, 29.2, 29.3, 29.5, 29.7, 31.8, 44.1, 59.6, 71.7, 101.6, 186.1, 193.8. Analysis Calculated for $\text{C}_{17}\text{H}_{29}\text{ClO}_2\text{S}$: C, 61.3, H, 8.78, Found: C, 61.9; H, 9.01.



5

(±)-4-(4-Chlorobutoxy)-5-methyl-5-hexyl-5H-thiophen-2-one (42). From 33 (36 mg, 0.17 mmol) and 3-iodo-1-chlorobutane (40 μL , 0.34 mmol) following general procedure H was obtained 42 (32 mg, 75%) after flash chromatography (20% EtOAc/Hexanes). ^1H NMR (400 MHz, CDCl_3) δ 0.86 (t, $J = 5.1$ Hz, 3 H), 1.09-1.14 (m, 1 H), 1.25 (s, 6 H), 1.44-1.53 (m, 1 H), 1.63 (s, 3 H), 1.77-1.85 (m, 2 H), 1.90-2.00 (m, 4 H), 3.59 (t, $J = 4.5$ Hz, 2 H), 3.95-3.99 (m, 2 H), 5.28 (s, 1 H). ^{13}C NMR (75 MHz, CDCl_3) δ 13.7, 22.3, 25.1, 26.1, 26.4, 29.1, 29.1, 31.5, 39.0, 43.9, 59.5, 71.6, 101.5, 185.9, 192.9. IR (NaCl) 2927, 1683, 1607 cm^{-1} . Analysis Calculated for $\text{C}_{15}\text{H}_{25}\text{ClO}_2\text{S}$: C, 59.1; H, 8.27; Found: C, 59.3; H, 8.39.

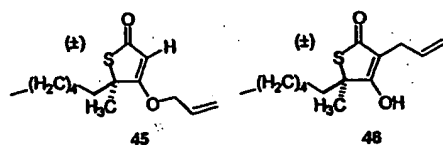
10



(±)-4-allyloxy-5-methyl-5-octyl-5H-thiophen-2-one (43). From 32 (31 mg, 0.12 mmol) and allyl bromide (21 μL , 0.25 mmol) following general procedure H was obtained a 3:1 mixture of 43 and 44 (26 mg, 74%) which could be separated and purified using flash chromatography (15% EtOAc/Hexanes). O-alkylated product 43. ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.3$ Hz, 3 H), 1.12-1.17 (m, 1 H), 1.24 (s, 10 H), 1.45-1.49 (m, 1 H), 1.64 (s, 3 H), 1.77-1.84 (m, 2 H), 4.47 (d, $J = 5.6$ Hz, 2 H), 5.29 (s, 1 H), 5.31 (d, $J = 11$ Hz, 1 H), 5.39 (d, $J = 17$ Hz, 1 H), 5.90-5.99 (ddd, $J = 5.6, 11, 17$ Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 22.6, 25.1, 26.5, 29.2, 29.3, 29.5, 31.8, 38.9, 59.7, 72.8, 102.0, 119.5, 130.8, 185.8, 193.8. IR (NaCl) 3441, 1681, 1609 cm^{-1} . Analysis Calculated for $\text{C}_{16}\text{H}_{26}\text{O}_2\text{S}$: C, 68.0, H, 9.20, Found: C, 68.1; H, 9.34.

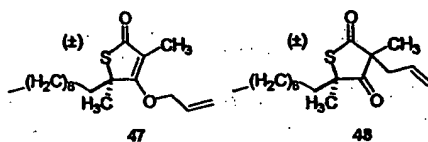
20

(44) C-alkylated product ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.5$ Hz, 3 H), 1.25 (m, 12 H), 1.54 (s, 3 H), 1.79-1.84 (m, 2 H), 2.43-2.47 (m, 2 H), 5.05-5.11 (m, 2 H), 5.57-5.69 (1 H).



5 (±)-4-allyloxy-5-methyl-5-hexyl-5H-thiophen-2-one (45). From 33 (270 mg, 1.3 mmol) and allyl bromide (0.2 mL, 2.52 mmol) following general procedure H, was obtained a 2.3:1 mixture of 45 and 46 (205 mg, 58%) which could be separated and purified using flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) (45) (O-alkylation) δ 0.84 (t, $J = 7$ Hz, 3 H), 1.09-1.17 (m, 1 H), 1.23 (s, 6 H), 1.40-1.51 (m, 1 H), 1.62 (s, 3 H), 1.73-1.83 (m, 2 H), 4.46 (d, $J = 5.6$ Hz, 2 H), 5.33 (d, $J = 10$ Hz, 1 H), 5.38 (d, $J = 17$ Hz, 1 H), 5.28 (s, 1 H), 5.87-5.98 (ddd, $J = 5.6, 10, 17$ Hz, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 22.5, 25.1, 26.5, 29.2, 31.6, 38.9, 59.7, 72.8, 101.9, 119.6, 130.7, 185.8, 193.9. Analysis Calculated for $\text{C}_{14}\text{H}_{22}\text{O}_2\text{S}$: C, 66.10; H, 8.72; Found: C, 66.04; H, 8.72.

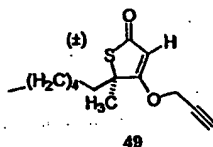
15 (46) (C-alkylation) ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.24 (bs, 8 H), 1.54 (s, 3 H), 1.81-1.84 (m, 2 H), 2.42-2.48 (m, 2 H), 5.05-5.10 (m, 2 H), 5.56-5.67 (m, 1 H).



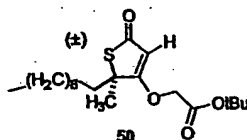
20 (±)-4-allyloxy-3,5-dimethyl-5-octyl-5H-thiophen-2-one (47). (±)-Allyl-3,5-Dimethyl-5-octyl-thiophene-2,4-dione (48). From 34 (70 mg, 0.27 mmol) and allyl bromide (47 μL , 0.55 mmol) following general procedure H, was obtained a 2.3:1 mixture of 47 and 48 (C-alkylation data not shown) (67 mg, 82 %) which could be separated and purified using flash chromatography (20% EtOAc/Hexanes).

(47). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.06-1.48 (m, 12 H), 1.58 (s, 3 H), 1.71-1.82 (m, 2 H), 1.94 (s, 3 H), 4.80-4.82 (m, 2 H), 5.28-5.46 (m, 2 H), 5.89-5.03 (m, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 9.65, 14.0, 22.6, 25.2, 26.6, 29.2, 29.3, 29.6, 31.8, 39.2, 57.5, 72.5, 118.2, 119.5, 132.6, 179.4, 193.8. IR (NaCl) 2855, 1676, 1628, 1580 cm^{-1} .

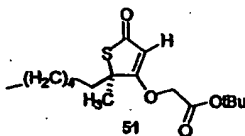
(48). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.16-1.47 (m, 15 H), 1.57 (s, 3 H), 1.74-1.96 (m, 2 H), 2.42-2.46 (m, 2 H), 5.04-5.10 (m, 2 H), 5.53-5.67 (m, 1 H).



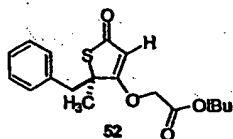
(±)-5-methyl-5-(prop-2-yn-1-yloxy)-2-oxo-thiophen-4-yl (49). From 33 (45 mg, 0.21 mmol) and propargyl bromide (37 μL , 0.21 mmol) following general procedure H was obtained 49 (21 mg, 40%). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.11-1.20 (m, 1 H), 1.24 (s, 6 H), 1.41-1.49 (m, 1 H), 1.63 (s, 3 H), 1.76-1.86 (m, 2 H), 2.59 (t, $J = 2.5$ Hz, 1 H), 4.62 (d, $J = 3.7$ Hz, 1 H), 4.63 (d, $J = 3.7$ Hz, 1 H), 5.43 (s, 1 H).



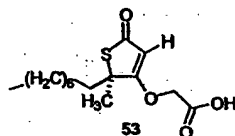
(±)-5-Methyl-5-(oct-2-yn-1-yloxy)-2-oxo-thiophen-4-yl (50). From 32 (60 mg, 0.25 mmol) and *tert*-butyl bromoacetate (73 μL , 0.49 mmol) following general procedure H, was obtained 50 (62 mg, 70%) after flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 7$ Hz, 3 H), 1.24 (s, 12 H), 1.49 (s, 9 H), 1.68 (s, 3 H), 1.83-1.86 (m, 2 H), 4.43 (s, 2 H), 5.19 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 22.6, 25.2, 26.3, 28.1, 29.2, 29.3, 29.5, 31.8, 38.9, 59.7, 68.5, 83.4, 102.1, 165.2, 185.5, 193.4. Analysis Calculated for $\text{C}_{19}\text{H}_{32}\text{O}_4\text{S}$: C, 64.0; H, 9.05; Found: C, 64.1; H, 9.08.



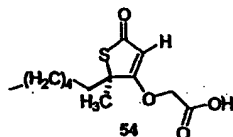
(±)-5-Methyl-5-(hex-2-yn-1-yloxy)-2-oxo-thiophen-4-yl (51). From 33 (169 mg, 0.79 mmol) and *tert*-butyl bromoacetate (0.23 mL, 1.58 mmol) following general procedure H, was obtained 51 (206 mg, 80%) after flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.82 (t, $J = 6.8$ Hz, 3 H), 1.21 (s, 8 H), 1.47 (s, 9 H), 1.64 (s, 3 H), 1.78-1.83 (m, 2 H), 4.41 (s, 2 H), 5.15 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 22.5, 25.1, 26.3, 28.0, 29.1, 31.5, 38.9, 59.6, 68.4, 83.4, 102.1, 165.2, 185.5, 193.4.



- 5 (±)- 5-Phenyl-5-methyl-2-oxo-thiophen-4-yloxy)-acetic acid *tert*-butyl ester (52). From 22 (150 mg, 0.68 mmol) and *tert*-butyl bromoacetate (0.20 mL, 1.36 mmol) following general procedure H, was obtained 52 (159 mg, 74 %) after flash chromatography (20% EtOAc/Hexanes). ¹H NMR (300 MHz, CDCl₃) δ 1.49 (s, 9 H), 1.69 (s, 3 H), 3.17 (s, 2 H), 4.44 (q, *J* = 8 Hz, 2 H), 5.13 (s, 1 H), 7.24 (m, 5 H); ¹³C NMR (75 MHz, CDCl₃) δ 25.8, 28.1, 45.0, 60.1, 68.4, 83.6, 102.6, 127.2, 128.1, 130.5, 135.9, 165.3, 184.9, 192.8.



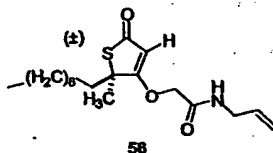
- 10 General Procedure I. (±)- 5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetic acid (53). To 50 (65 mg, 0.18 mmol) dissolved in CH₂Cl₂ (1.4 mL) was added trifluoroacetic acid (TFA) (0.7 mL) and the solution was stirred at room temperature for 4 hours. The solvents were evaporated and the crude material was chromatographed (20% EtOAc/2% CH₃CO₂H/Hexanes) to give pure 53 (48 mg, 89%). ¹H NMR (300 MHz, CDCl₃) δ 0.86 (t, *J* = 6.9 Hz, 3 H), 1.24 (s, 11 H), 1.47-1.48 (m, 1 H), 1.68 (s, 3 H), 1.84-1.88 (m, 2 H), 4.62 (s, 2 H), 5.31 (s, 1 H); ¹³C NMR (75 MHz, CDCl₃) δ 14.1, 22.6, 25.1, 26.1, 29.2, 29.3, 29.5, 31.8, 38.9, 60.1, 67.7, 102.4, 169.8, 185.8, 195.4. IR (NaCl) 3442, 1645 cm⁻¹; Analysis Calculated for C₁₅H₂₄O₄S: C, 59.9; H, 8.05; Found: C, 60.0; H, 8.09.
- 15



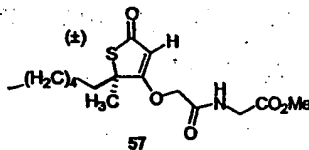
- 20 (±)- 5-Methyl-5-hexyl-2-oxo-thiophen-4-yloxy)-acetic acid (54). To 51 (177 mg, 0.54 mmol) and trifluoroacetic acid (TFA) (2.61 mL) following general procedure I was obtained 54 (144 mg, 98%) after flash chromatography (20% EtOAc/2% CH₃CO₂H/ Hexanes). ¹H NMR (300 MHz, CDCl₃) δ 0.85 (t, *J* = 6.8 Hz, 3 H), 1.24 (s, 7 H), 1.44-1.47 (m, 1 H), 1.68 (s, 3 H), 1.84-1.91 (m,

CC1=C(C(=O)OCC1C(=O)S1=CC=CC=C1)OC(=O)O

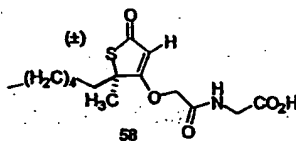
10



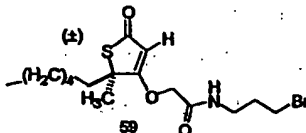
37



General Procedure J. (±)-(5-methyl-5-hexyl-2-oxo-thiophen-4-yloxy)-alkynyl-methyl glycinate (57). To a solution of 54 (42.4 mg, 0.15 mmol) in CH₃CN (0.86 mL) was added tris(2-oxo-3-oxazoliny)phosphine oxide³ (91 mg, 0.20 mmol), methylglycinate hydrochloride (19.7 mg, 0.16 mmol) and NEt₃ (43 µL, 0.31 mmol) and the solution was allowed to stir at room temperature for 20 minutes. The mixture was poured into a solution of NH₄Cl_(sat)/1 N HCl (10 mL) and extracted with Et₂O (3 x 10 mL). The combined organics were dried (MgSO₄), filtered, evaporated and chromatographed (40-50% EtOAc/Hexanes) to give pure 57 (43 mg, 80%). ¹H NMR (300 MHz, CDCl₃) δ 0.85 (t, *J* = 6.8 Hz, 3 H), 1.23-1.26 (m, 7 H), 1.49-1.55 (m, 1 H), 1.65 (s, 3 H), 1.84-1.90 (m, 2 H), 3.79 (s, 3 H), 4.11 (d, *J* = 5 Hz, 1 H), 4.12 (d, *J* = 5 Hz, 1 H), 4.47 (s, 2 H), 5.36 (s, 1 H), 6.76 (bs, 1 H).

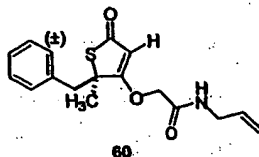


(±)-(5-methyl-5-hexyl-2-oxo-thiophen-4-yloxy)-alkynyl glycinate (58). To 57 (22 mg, 0.06 mmol) dissolved in THF/H₂O (0.5 mL, 3:1), cooled to 0 °C was added LiOH (3 mg, 0.07 mmol) and this solution was allowed to stir for 45 minutes. Then the mixture was poured into a solution of HCl (10 mL, 1 N) and extracted with Et₂O (3 x 10 mL). The combined organics were dried (MgSO₄), filtered and evaporated to give crude 58. Flash chromatography (50% EtOAc/2%CH₃CO₂H/Hexanes) gave pure 58 (19 mg, 86%). ¹H NMR (300 MHz, CDCl₃) δ 0.85 (t, *J* = 6.7 Hz, 3 H), 1.25 (s, 7 H), 1.48-1.52 (m, 2 H), 1.68 (s, 3 H), 2.08-2.10 (m, 2 H), 4.05 (s, 2 H), 4.56 (s, 2 H), 5.41 (s, 1 H).

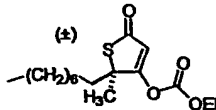


(±)-N-(4-Bromobutyl)-(5-methyl-5-hexyl-2-oxo-thiophen-4-yloxy)-acetamide (59). To 54 (61 mg, 0.22 mmol) and 1-aminopropanol hydrobromide (50 mg, 0.23 mmol) following general

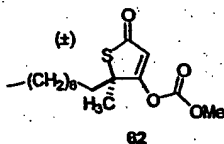
procedure J gave 59 (65 mg, 74%) after flash chromatography (50% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.9$ Hz, 3 H), 1.12-1.15 (m, 1 H), 1.23-1.28 (s, 6 H), 1.46-1.53 (m, 1 H), 1.69 (s, 3 H), 1.82-1.88 (m, 2 H), 2.14 (quint, $J = 6$ Hz, 2 H), 3.42 (m, 2 H), 3.54 (q, $J = 6.3$ Hz, 2 H), 4.43 (s, 2 H), 5.35 (s, 1H) 6.45 (bs, 1 H).



5 (±) N-allyl-(5-phenyl-5-methyl-2-oxo-thiophen-4-yloxy)-acetamide (60). To 55 (72 mg, 0.26 mmol) and allyl amine (21 μL , 0.28 mmol) following general procedure J gave 60 (39 mg, 47 %) after flash chromatography (gradient 10-50% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 1.73 (s, 3 H), 3.17 (s, 2 H), 3.93 (m, 2 H), 4.41 (s, 2 H), 5.22 (m, 2 H), 5.24 (s, 1 H), 5.80 (m, 1
10 H), 5.83 (s, 1 H), 7.24 (m, 5 H) ^{13}C NMR (75 MHz, CDCl_3) δ 26.0, 41.6, 45.4, 59.7, 70.3, 103.9, 117.1, 127.5, 128.3, 130.2, 133.3, 135.6, 165.3, 183.4, 192.0.

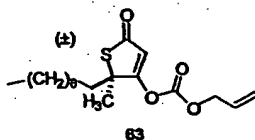


General Procedure K. (±)-4-Carbonic acid ethyl ester-5-methyl-5-octyl-5H-thiophen-2-
15 one (61). To a solution of 32 (95 mg, 0.39 mmol) in THF (1.8 mL) cooled to -78°C was added LiHMDS (0.58 mL, 0.58 mmol, 1 M in THF) and the solution was allowed to stir for 30 minutes at -78°C . Ethyl chloroformate (60 μL , 0.62 mmol) was then added and the mixture was transferred to an ice bath and then allowed to slowly warm to room temperature. After 1 hour at
room temperature the mixture was poured into a solution of HCl (1 N)/ NH_4Cl (sat) (10 mL) and
20 extracted with Et_2O (3 x 10 mL). The combined organics were dried (MgSO_4), filtered, evaporated and chromatographed (20% EtOAc/Hexanes) to give pure 61 (111 mg, 91%). ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t, $J = 6.9$ Hz, 3 H), 1.12-1.17 (m, 11 H), 1.38 (t, $J = 7$ Hz, 3 H), 1.42-1.50 (m, 1 H), 1.67 (s, 3 H), 1.82 (d, $J = 9$ Hz, 1 H), 1.85 (d, $J = 9$ Hz, 1 H), 4.33 (q, $J = 7$ Hz, 2 H), 6.38 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.0, 14.0, 22.6, 25.2, 25.8, 29.1, 29.2,
25 20.4, 31.8, 38.4, 60.1, 66.0, 112.8, 150.2, 175.6, 193.9. IR (NaCl) 2928, 1782, 1690, 1625 cm^{-1} .
Analysis Calculated for $\text{C}_{16}\text{H}_{26}\text{O}_4\text{S}$: C, 61.1; H, 8.33; Found: C, 61.5; H, 8.32.



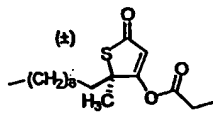
62

(±)-4-Carbonic acid methyl ester- 5-methyl-5-octyl-5 *H*- thiophen-2-one (62). From 32 (73 mg, 0.30 mmol) and methyl chloroformate (37 μ L, 0.48 mmol) following general procedure K was obtained 62 (63 mg, 70 %) after flash chromatography (20% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t, $J = 7$ Hz, 3 H), 1.15-1.21 (m, 1 H), 1.22 (s, 10 H), 1.41-1.51 (m, 1 H), 1.66 (s, 3 H), 1.81 (d, $J = 9$ Hz, 1 H), 1.83 (d, $J = 9$ Hz, 1 H), 3.92 (s, 3 H), 6.39 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 22.6, 25.2, 25.9, 29.2, 29.3, 29.4, 31.8, 38.4, 56.2, 60.2, 112.9, 150.9, 175.5, 194.1. IR (NaCl) 3382, 1626, 1560, 1542 cm^{-1} . Analysis Calculated for $\text{C}_{15}\text{H}_{24}\text{O}_4\text{S}$: C, 59.9; H, 8.05; Found: C, 60.3; H, 8.10.



63

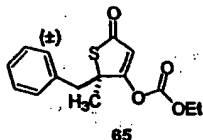
(±)-4-Carbonic acid allyl ester- 5-methyl-5-octyl-5 *H*-thiophen-2-one (63). From 32 (51.5 mg, 0.21 mmol) and allyl chloroformate (33 μ L, 0.32 mmol) following general procedure K was obtained 63 (46.3 mg, 67%) after flash chromatography (15% EtOAc/Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t, $J = 7$, 3 H), 1.16-1.23 (bs, 10 H), 1.41-1.51 (m, 2 H), 1.67 (s, 3 H), 1.81-1.87 (m, 2 H), 4.74 (app dt, $J = 6$, 1.3 Hz, 2 H), 5.37 (app dq, $J = 10.3$, 1.02 Hz, 1 H), 5.44 (app dq, $J = 15.9$, 1.02 Hz, 1 H), 5.90-6.0 (m, 1 H), 6.39 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) 14.0, 22.6, 25.2, 25.8, 29.1, 29.2, 29.4, 31.8, 38.4, 60.1, 70.2, 112.9, 120.6, 130.23, 150.0, 175.5, 193.7. IR (NaCl) 2927, 1782, 1691, 1606 cm^{-1} . Analysis Calculated for $\text{C}_{17}\text{H}_{26}\text{O}_4\text{S}$: C, 62.5; H, 8.03; Found: C, 62.6; H, 8.07.



64

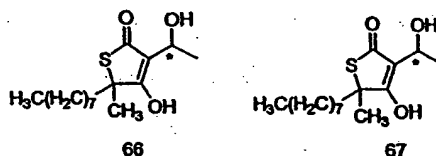
(±)-4-Propionyl- 5-methyl-5-octyl-5 *H*- thiophen-2-one (64). From 32 (40 mg, 0.17mmol) and propionyl chloride (20 μ L, 0.22mmol) following general procedure K was obtained 64 (23.1 mg, 47%) after flash chromatography (15% EtOAc/ Hexanes). ^1H NMR (300 MHz, CDCl_3) δ 0.85 (t,

$J = 7$ Hz, 3 H), 1.12-1.25 (m, 13 H), 1.42-1.49 (m, 2 H), 1.64 (s, 3 H), 1.78-1.84 (m, 2 H), 2.57 (q, $J = 7.5$ Hz, 2 H), 6.39 (s, 1 H); ^{13}C NMR (75 MHz, CDCl_3) δ 8.71, 14.0, 22.6, 25.1, 25.9, 27.9, 29.1, 29.3, 29.5, 31.8, 38.6, 60.4, 113.8, 169.1, 177.0, 179.9. IR (NaCl) 2928, 1787, 1688 cm^{-1} ; Analysis Calculated for $\text{C}_{16}\text{H}_{26}\text{O}_3\text{S}$: C, 64.4; H, 8.78; Found: C, 64.3; H, 8.89.



5 (±)-4-carbonic acid ethyl-ester-5-phenyl-5-methyl-5H-thiophen-2-one (65). From 22 (50 mg, 0.23 mmol) and ethyl chloroformate (35 μL , 0.36 mmol) following general procedure K was obtained 65 (67 mg, 88%). ^1H NMR (300 MHz, CDCl_3) δ 1.31 (t, $J = 7$ Hz, 3 H), 1.69 (s, 3 H), 3.15 (s, 2 H), 4.36 (q, $J = 7$ Hz, 2 H), 6.33 (s, 1 H), 7.18-7.27 (m, 5 H); ^{13}C NMR (75 MHz, CDCl_3) δ 14.1, 25.3, 44.6, 60.6, 66.2, 113.2, 127.4, 128.2, 130.3, 135.4, 150.1, 175.1, 193.3.

10



4-Hydroxy-3-(1-hydroxyethyl)-5-methyl-5-octyl-5H-thiophen-2-one. (66, 67). To 32 (247 mg, 1.02 mmol) dissolved in hexanes was added triethylamine (0.23 mL, 1.68 mmol) and trimethylsilylchloride (0.21 mL, 1.64 mmol) and the solution was allowed to stir at room temperature for 4 h. The mixture was filtered over celite and evaporated to provide 5-methyl-5-octyl-4-trimethylsilanyloxy-5H-thiopen-2-one. To a solution of TiCl_4 (0.7 mL, 0.7 mmol) in CH_2Cl_2 (1.95 mL) at -78°C was added acetaldehyde (54 μL , 0.97 mmol) and this solution was allowed to stir for 5 min at -78°C . Then, 5-methyl-5-octyl-4-trimethylsilanyloxy-5H-thiopen-2-one dissolved in CH_2Cl_2 (0.4 mL) was cannulated into TiCl_4 /acetaldehyde solution giving a bright orange color. This mixture was allowed to warm and stir for 20 min at 0°C . The mixture was poured into $\text{NH}_4\text{Cl}_{(\text{sat})}$ (15 mL) and extracted with CH_2Cl_2 (3 x 15 mL). The organics were combined, dried (MgSO_4), filtered and evaporated. Flash chromatography (10% EtOAc/Hexanes) provided pure 66 (34 mg) and 67 (24 mg) (50%). (66) ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.9$ Hz, 3 H), 1.05-1.08 (m, 1 H), 1.24 (bs, 11 H), 1.49 (d, $J = 6.5$ Hz, 3 H,

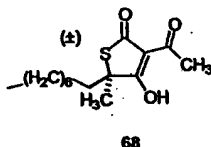
15

20

25

rotamer) 1.55 (d, $J = 5.2$ Hz, 3 H, rotamer), 1.62 (s, 3 H), 1.78-1.82 (m, 2 H), 4.68 (q, $J = 6.5$ Hz, 1 H, rotamer), 5.04 (q, $J = 5.2$ Hz, 1 H, rotamer). HRMS (ES) m/z calculated for $C_{16}H_{28}O_3SNa^+$ ($M+CH_2+Na^+$) 323.1660 obsd. 323.1660.

- 5 (67) 1H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, $J = 6.9$ Hz, 3 H), 1.24 (bs, 12 H), 1.47 (d, $J = 6.6$ Hz, 3 H, rotamer), 1.54 (d, $J = 5.4$ Hz, 3 H, rotamer), 1.59 (s, 3 H), 1.76-1.82 (m, 2 H), 4.65 (q, $J = 6.3$ Hz, 1 H), 5.06 (q, $J = 5.4$ Hz, 1 H). HRMS (ES) m/z calculated for $C_{16}H_{28}O_3SNa^+$ ($M+CH_2+Na^+$) 323.1660 obsd. 323.1660.



10

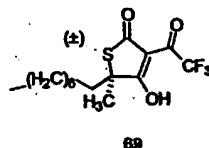
General Procedure L. 3-Alkynyl-4-hydroxy-5-methyl-5-octyl-5H-thiophen-2-one. (68). To

32 (94 mg, 0.38 mmol) in CH_2Cl_2 (1.9 mL) at 0 °C was added NEt_3 (58 μ L, 0.42 mmol), dimethylaminopyridine (DMAP) (19 mg, 0.15 mmol) and acetic anhydride (43 μ L, 0.47 mmol).

The solution stirred at 0 °C for 15 min then was allowed to warm and stir at room temperature for

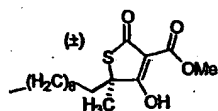
- 15 2-14 h or until TLC indicated completion of the reaction. The mixture was poured into NH_4Cl (sat)/HCl (1 N) (3:1, 8 mL) and extracted with CH_2Cl_2 (3 x 10 mL). The organics were combined, dried ($MgSO_4$), filtered and evaporated to give crude 68. Flash chromatography 30%EtOAc/2%AcOH/Hex ($r_f = 0.44$) gave pure 68 (83 mg, 78%). 1H NMR (300 MHz, $CDCl_3$) δ 0.84 (m, 3 H), 1.22 (bs, 10 H), 1.48 (m, 2 H), 1.65 (s, 3 H), 1.77-1.92 (m, 2 H), 2.55 (s, 3 H).

- 20 ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 22.6, 23.8, 25.1, 26.3, 29.1, 29.2, 29.5, 31.7, 39.4, 59.7, 109.7, 190.5, 195.5, 204.9. HRMS (EI) m/z calculated for $C_{15}H_{24}O_3S^+$ (M^+) 284.1441 obsd. 284.1414.



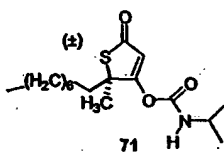
- 25 **4-Hydroxy-5-methyl-5-octyl-3-(2,2,2-trifluoroalkynyl)-5H-thiophen-2-one. (69).** To 32 (90 mg, 0.37 mmol), trifluoroacetic anhydride (114 μ L, 0.81 mmol), dimethylaminopyridine

(DMAP) (18 mg, 0.15 mmol) and NEt_3 (108 μL , 0.77 mmol) following General Procedure L was obtained 69 (107 mg, 86%) after flash chromatography (40%Hex/10% THF/2%AcOH/EtOAc). ^1H NMR (300 MHz, MeOD) δ 0.85 (t, $J = 6.9$ Hz, 3 H), 1.09 (m, 1 H), 1.21 (bs, 11 H), 1.38 (s, 3 H), 1.51-1.60 (m, 1 H), 1.65-1.71 (m, 1 H). HRMS (EI) m/z calculated for $\text{C}_{15}\text{H}_{21}\text{F}_3\text{O}_3\text{S}^+$ (M^+) 338.1158 obsd. 338.1171.



70

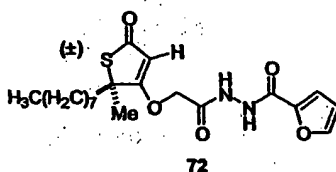
4-Hydroxy-5-methyl-5-octyl-2-oxo-2,5-dihydro-thiophene-3-carboxylic acid methyl ester (70). To 32 (91 mg, 0.37 mmol), methyl chloroformate (63 μL , 0.81 mmol), dimethylaminopyridine (DMAP) (23 mg, 0.18 mmol) and NEt_3 (108 μL , 0.77 mmol) following General Procedure L was obtained 70 (66 mg, 59%, 79% based on recovered starting material) after flash chromatography (30% EtOAc/2%AcOH/Hexanes-10%THF/2%AcOH/EtOAc). ^1H NMR (300 MHz, MeOD) δ 0.86 (t, $J = 6.9$ Hz, 3 H), 1.20 (bs, 12 H), 1.35 (s, 3 H), 1.55 (m, 1 H), 1.71-1.75 (m, 1 H), 3.59 (s, 3 H); ^{13}C NMR (75 MHz, MeOD) δ 13.3, 21.8, 24.4, 27.0, 28.5, 28.6, 29.0, 30.2, 31.0, 50.4, 58.3, 124.6, 168.1, 187.7, 196.7. HRMS (EI) m/z calculated for $\text{C}_{15}\text{H}_{24}\text{O}_4\text{S}^+$ (M^+) 300.1389 obsd. 300.1375.



71

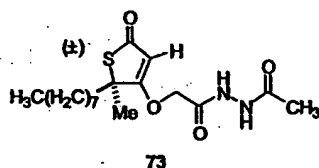
Isopropyl-carbamic acid 2-methyl-2-octyl-5-oxo-2,5-dihydro-thiophen-3-yl ester (71). To 32 (46 mg, 0.19 mmol) dissolved in hexanes was added triethylamine (43 μL , 0.31 mmol) and trimethylsilylchloride (36 μL , 0.29 mmol) and the solution was allowed to stir at room temperature for 4 h. The mixture was filtered over celite and evaporated to provide 5-methyl-5-octyl-4-trimethylsilyloxy-5-H-thiopen-2-one which was redissolved in CH_2Cl_2 (0.4 mL). To this mixture was added isopropyl isocyanate (19.2 mL, 0.19 mmol) and the solution was allowed to stir at room temperature for 2 hours. $\text{NH}_4\text{Cl}(\text{sat})$ (5 mL) was added and the mixture was extracted with CH_2Cl_2 (3 x 10 mL). The organics were combined, dried (MgSO_4), filtered and

evaporated to give crude 71. Flash chromatography (20% EtOAc/ 2% AcOH/Hexanes) gave pure 71 (35 mg, 60%). ¹H NMR (300 MHz, CDCl₃) δ 0.85 (t, *J* = 7.0 Hz, 3 H), 1.14-1.24 (m, 17 H), 1.45 (m, 1 H), 1.63 (s, 3 H), 1.76-1.79 (m, 2 H), 3.81-3.88 (m, 1 H), 5.16 (d, *J* = 7 Hz, 1 H), 6.33 (s, 1 H). ¹³C NMR (75 MHz, CDCl₃) δ 13.9, 20.4, 22.5, 22.8, 25.1, 25.9, 29.1, 29.3, 29.5, 31.8, 38.7, 44.0, 60.2, 111.6, 149.7, 176.2, 194.5.

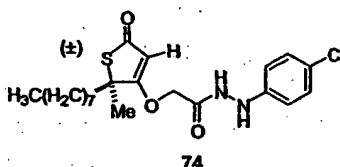


General Procedure M. (±)-(5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetic acid-N'-(2-furoic)-hydrazide (72). To a cooled solution (0 °C) of 53 (100 mg, 0.33 mmol) in CH₂Cl₂ (1.61 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC) (128 mg, 0.43 mmol), DMAP (6.0 mg, 0.05 mmol), and 2-furoic hydrazide (54 mg, 0.43 mmol). This mixture stirred at 0 °C for 30 minutes, then was allowed to warm to room temperature and stir for 12 h. The solution was poured into NH₄Cl (10 ml, sat) and extracted with CH₂Cl₂ (3 x 10 ml). The combined organics were dried (Na₂SO₄), filtered and evaporated to give crude 72.

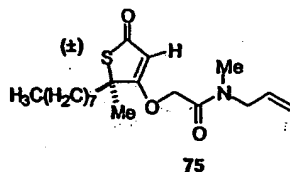
Flash chromatography (10% EtOAc/Hex) gave pure 72 (91 mg, 68%). ¹H NMR (400 MHz, CDCl₃) δ 0.84 (t, *J* = 6.6 Hz, 3 H), 1.21 (m, 11 H), 1.43-1.47 (m, 1 H), 1.66 (s, 3 H), 1.81-1.86 (m, 2 H), 4.64 (s, 2 H), 5.42 (s, 1 H), 6.47 (dd, *J* = 1.6, 3.6 Hz, 1 H), 7.16 (d, *J* = 4 Hz, 1 H), 7.45 (m, 1 H), 9.32 (d, *J* = 4 Hz, 1 H), 9.44 (d, *J* = 4 Hz, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 14.0, 22.6, 25.3, 26.0, 29.2, 29.3, 29.5, 31.7, 38.8, 59.7, 69.1, 103.0, 112.3, 116.5, 145.1, 145.4, 156.4, 164.2, 184.8, 193.9.



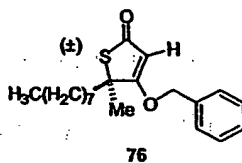
(±)-(5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetic-acid-N'-acetylhydrazide (73). To 53 (100 mg, 0.33 mmol) and acetic hydrazide (26.8 mg, 0.36 mmol) following General Procedure M was obtained 73 (70.4 mg, 60%) after flash chromatography (2% AcOH/EtOAc). ¹H NMR (400 MHz, CDCl₃) δ 0.85 (t, *J* = 7.2 Hz, 3 H), 1.23 (m, 11 H), 1.48-1.52 (m, 1 H), 1.67 (s, 3 H), 1.84-1.86 (m, 2 H), 2.07 (s, 3 H), 4.64 (s, 2 H), 5.42 (s, 1 H). ¹³C NMR (100 MHz, CDCl₃) δ 14.1, 20.6, 22.6, 25.2, 26.0, 29.2, 29.3, 29.5, 31.8, 38.8, 59.8, 68.9, 102.9, 163.1, 168.1, 184.9, 194.2.



10 (±)-(5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetic-acid-N'-(4-chloro-phenyl)-hydrazide (74) To 53 (100 mg, 0.33 mmol) and 4-chlorophenylhydrazine hydrochloride (76.8 mg, 0.43 mmol) following General Procedure M was obtained 74 (74 mg, 53%) after flash chromatography (50% EtOAc/Hex). ¹H NMR (300 MHz, CDCl₃) δ 0.86 (t, *J* = 6 Hz, 3 H), 1.24 (m, 11 H), 1.46-1.54 (m, 1 H), 1.71 (s, 3 H), 1.82-1.90 (m, 2 H), 4.57 (s, 2 H), 5.39 (s, 1 H), 6.75 (d, *J* = 8.8 Hz, 2 H), 7.18 (d, *J* = 8.8 Hz, 2 H), 7.38 (s, 1 H), 8.09 (s, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 14.1, 22.6, 25.3, 26.1, 29.2, 29.3, 29.5, 31.8, 38.8, 59.7, 69.7, 103.2, 114.7, 126.4, 145.8, 129.2, 165.9, 184.3, 193.5. IR (NaCl) 2957, 1695, 1658, 1609 cm⁻¹.



(±)-*N*-Allyl-*N*-methyl-(5-Methyl-5-octyl-2-oxo-thiophen-4-yloxy)-acetamide (75). To 53 (83 mg, 0.28 mmol) and *N*-methyl-*N*-allylamine (29 μ L, 0.30 mmol) following General Procedure M was obtained 75 (51 mg, 52%) after flash chromatography (40% EtOAc/Hex). ^1H NMR (300 MHz, CDCl_3) δ 0.83 (t, $J = 6.9$ Hz, 3 H), 1.22 (m, 11 H), 1.43-1.47 (m, 1 H), 1.67 (s, 3 H), 1.82-1.86 (m, 2 H), rotamer 1: 2.91 (s, 3 H), rotamer 2: 2.95 (s, 3 H), rotamer 1: 3.84 (d, $J = 4.8$ Hz, 2 H), rotamer 2: 3.98 (d, $J = 6$ Hz, 2 H), rotamer 1: 4.62 (s, 2 H), rotamer 2: 4.65 (s, 2 H), 5.12-5.28 (m, 2 H), rotamer 1: 5.18 (s, 1 H), rotamer 2: 5.25 (s, 1 H), 5.65-5.81 (m, 1 H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.0, 22.5, 25.1, 26.2, 29.1, 29.3, 29.4, 31.7, 33.4 (rotamer 2: 33.9), 38.8, 50.2 (rotamer 2: 51.0), 59.7, 69.0 (rotamer 2: 69.3), 102.3, 117.4 (rotamer 2: 118.2), 131.6 (rotamer 2: 131.8), 164.5 (rotamer 2: 164.9), 185.5 (rotamer 2: 185.6), 193.4.



(±)-4-Benzoyloxy-3,5-dimethyl-5-octyl-5-H-thiophen-2-one (76). To 32 (50 mg, 0.21 mmol) and benzyl bromide (37 mL, 0.31 mmol) following General Procedure H, was obtained 76 (49 mg, 75%) after flash chromatography (15% EtOAc/Hex). ^1H NMR (300 MHz, CDCl_3) δ 0.86 (t, $J = 6.9$ Hz, 3 H), 1.24 (m, 11 H), 1.41-1.48 (m, 1 H), 1.66 (s, 3 H), 1.79-1.86 (m, 2 H), 4.98 (s, 2 H), 5.39 (s, 1 H), 7.31-7.42 (m, 5 H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 22.6, 25.0, 26.4, 29.1, 29.3, 29.4, 31.8, 38.8, 59.7, 74.0, 102.2, 127.6, 128.8, 128.8, 134.3, 185.8, 194.1. IR (NaCl) 2928, 1681, 1610 cm^{-1} .

References:

1. Strijtveen, B.; Kellogg, R. M. *Tetrahedron*. 1987, 43, 5039-5054.
- 5 2. Sasaki, H.; Oishi, H.; Hayashi, T.; Matsuura, I.; Ando K.; Sawada, M. *J. Antibiotics* 1982, 3
3. Kunieda, T.; Nagamatsu, T.; Higuchi, T.; Hirobe, M. *Tetrahedron Lett.* 1988, 29, 2203-2206.

10 BIOLOGICAL AND BIOCHEMICAL METHODS***Purification of FAS from ZR-75-1 Human Breast Cancer Cells.***

Human FAS was purified from cultured ZR-75-1 human breast cancer cells obtained from the American Type Culture Collection. The procedure, adapted from Linn *et al.*, 1981, and Kuhajda *et al.*, 1994, utilizes hypotonic lysis, successive polyethyleneglycol (PEG) precipitations, and anion exchange chromatography. ZR-75-1 cells are cultured at 37 °C with 5% CO₂ in RPMI culture medium with 10% fetal bovine serum, penicillin and streptomycin.

Ten T150 flasks of confluent cells are lysed with 1.5 ml lysis buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride (PMSF), 0.1% Igepal CA-630) and dounce homogenized on ice for 20 strokes. The lysate is centrifuged in JA-20 rotor (Beckman) at 20,000 rpm for 30 minutes at 4 °C and the supernatant is brought to 42 ml with lysis buffer. A solution of 50% PEG 8000 in lysis buffer is added slowly to the supernatant to a final concentration of 7.5%. After rocking for 60 minutes at 4 °C, the solution is centrifuged in JA-20 rotor (Beckman) at 15,000 rpm for 30 minutes at 4 °C. Solid PEG 8000 is then added to the supernatant to a final concentration of 15%. After the rocking and centrifugation is repeated as above, the pellet is resuspended overnight at 4 °C in 10 ml of Buffer A (20 mM K₂HPO₄, pH 7.4). After 0.45 µM filtration, the protein solution is applied to a Mono Q 5/5 anion exchange column (Pharmacia). The column is washed for 15 minutes with buffer A at 1 ml/minute, and

bound material is eluted with a linear 60-ml gradient over 60 minutes to 1 M KCl. FAS (MW~270 kD) typically elutes at 0.25 M KCl in three 0.5 ml fractions identified using 4-15% SDS-PAGE with Coomassie G250 stain (Bio-Rad). FAS protein concentration is determined using the Coomassie Plus Protein Assay Reagent (Pierce) according to manufacturer's specifications
5 using BSA as a standard. This procedure results in substantially pure preparations of FAS (>95%) as judged by Coomassie-stained gels.

Measurement of FAS Enzymatic Activity and Determination of the IC₅₀ of the Compounds

FAS activity is measured by monitoring the malonyl-CoA dependent oxidation of
10 NADPH spectrophotometrically at OD₃₄₀ in 96-well plates (Dils *et al* and Arslanian *et al*, 1975). Each well contains 2 µg purified FAS, 100 mM K₂HPO₄, pH 6.5, 1 mM dithiothreitol (Sigma), and 187.5 µM β-NADPH (Sigma). Stock solutions of inhibitors are prepared in DMSO at 2, 1, and 0.5 mg/ml resulting in final concentrations of 20, 10, and 5 µg/ml when 1 µl of stock is added per well. For each experiment, cerulenin (Sigma) is run as a positive control along with
15 DMSO controls, inhibitors, and blanks (no FAS enzyme) all in duplicate.

The assay is performed on a Molecular Devices SpectraMax Plus Spectrophotometer. The plate containing FAS, buffers, inhibitors, and controls are placed in the spectrophotometer heated to 37°C. Using the kinetic protocol, the wells are blanked on duplicate wells containing 100 µl of 100 mM K₂HPO₄, pH 6.5 and the plate is read at OD₃₄₀ at 10 sec
20 intervals for 5 minutes to measure any malonyl-CoA independent oxidation of NADPH. The plate is removed from the spectrophotometer and malonyl-CoA (67.4 µM, final concentration per well) and alkynyl-CoA (61.8 µM, final concentration per well) are added to each well except to the blanks. The plate is read again as above with the kinetic protocol to measure the malonyl-

CoA dependent NADPH oxidation. The difference between the ΔOD_{340} for the malonyl-CoA dependent and non-malonyl-CoA dependent NADPH oxidation is the specific FAS activity. Because of the purity of the FAS preparation, non-malonyl-CoA dependent NADPH oxidation is negligible.

- 5 The IC_{50} for the compounds against FAS is determined by plotting the ΔOD_{340} for each inhibitor concentration tested, performing linear regression and computing the best-fit line, r^2 values, and 95% confidence intervals. The concentration of compound yielding 50% inhibition of FAS is the IC_{50} . Graphs of ΔOD_{340} versus time are plotted by the SOFTmax PRO software (Molecular Devices) for each compound concentration. Computation of linear
- 10 regression, best-fit line, r^2 , and 95% confidence intervals are calculated using Prism Version 3.0 (Graph Pad Software).

Crystal Violet Cell Growth Assay

- The crystal violet assay measure cell growth but not cytotoxicity. This assay
- 15 employs crystal violet staining of fixed cells in 96-well plates with subsequent solubilization and measurement of OD_{490} on a spectrophotometer. The OD_{490} corresponds to cell growth per unit time measured. Cells are treated with the compounds of interest or vehicle controls and IC_{50} for each compound is computed.

- To measure the cytotoxicity of specific compounds against cancer cells, 5×10^4
- 20 MCF-7 human breast cancer cells, obtained from the American Type Culture Collection are plated per well in 24 well plates in DMEM medium with 10% fetal bovine serum, penicillin, and streptomycin. Following overnight culture at 37°C and 5% CO_2 , the compounds to be tested, dissolved in DMSO, are added to the wells in 1 μ l volume at the following concentrations: 50,

40, 30, 20, and 10 µg/ml in triplicate. Additional concentrations are tested if required. 1 µl of DMSO is added to triplicate wells are the vehicle control. C75 is run at 10, and 5 µg/ml in triplicate as positive controls.

After 72 hours of incubation, cells are stained with 0.5 ml of Crystal Violet stain
5 (0.5% in 25% methanol) in each well. After 10 minutes, wells are rinsed, air dried, and then solubilized with 0.5 ml 10% sodium dodecylsulfate with shaking for 2 hours. Following transfer of 100 µl from each well to a 96-well plate, plates are read at OD₄₉₀ on a Molecular Devices SpectraMax Plus Spectrophotometer. Average OD₄₉₀ values are computed using SOFTmax Pro Software (Molecular Devices) and IC₅₀ values are determined by linear regression analysis using
10 Prism version 3.02 (Graph Pad Software, San Diego).

XTT Cytotoxicity Assay

The XTT assay is a non-radioactive alternative for the [⁵¹Cr] release cytotoxicity assay. XTT is a tetrazolium salt that is reduced to a formazan dye only by metabolically active,
15 viable cells. The reduction of XTT is measured spectrophotometrically as OD₄₉₀ – OD₆₅₀.

To measure the cytotoxicity of specific compounds against cancer cells, 9×10^3 MCF-7 human breast cancer cells, obtained from the American Type Culture Collection are plated per well in 96 well plates in DMEM medium with 10% fetal bovine serum, insulin, penicillin, and streptomycin. Following overnight culture at 37°C and 5% CO₂, the compounds
20 to be tested, dissolved in DMSO, are added to the wells in 1 µl volume at the following concentrations: 80, 40, 20, 10, 5, 2.5, 1.25, and 0.625 µg/ml in triplicate. Additional concentrations are tested if required. 1 µl of DMSO is added to triplicate wells are the vehicle control. C75 is run at 40, 20, 10, 15, 12.5, 10, and 5 µg/ml in triplicate as positive controls.

After 72 hours of incubation, cells are incubated for 4 hours with the XTT reagent as per manufacturer's instructions (Cell Proliferation Kit II (XTT) Roche). Plates are read at OD₄₉₀ and OD₆₅₀ on a Molecular Devices SpectraMax Plus Spectrophotometer. Three wells containing the XTT reagent without cells serve as the plate blank. XTT data are reported as OD₄₉₀ - OD₆₅₀. Averages and standard error of the mean are computed using SOFTmax Pro software (Molecular Dynamics).

The IC₅₀ for the compounds is defined as the concentration of drug leading to a 50% reduction in OD₄₉₀ - OD₆₅₀ compared to controls. The OD₄₉₀ - OD₆₅₀ are computed by the SOFTmax PRO software (Molecular Devices) for each compound concentration. IC₅₀ is calculated by linear regression, plotting the FAS activity as percent of control versus drug concentrations. Linear regression, best-fit line, r^2 , and 95% confidence intervals are determined using Prism Version 3.0 (Graph Pad Software).

15 **Measurement of [¹⁴C]acetate Incorporation into Total Lipids and Determination of IC₅₀ of Compounds**

This assay measures the incorporation of [¹⁴C]acetate into total lipids and is a measure of fatty acid synthesis pathway activity *in vitro*. It is utilized to measure inhibition of fatty acid synthesis *in vitro*.

MCF-7 human breast cancer cells cultured as above, are plated at 5×10^4 cells per well in 24-well plates. Following overnight incubation, the compounds to be tested, solubilized in DMSO, are added at 5, 10, and 20 $\mu\text{g/ml}$ in triplicate, with lower concentrations tested if necessary. DMSO is added to triplicate wells for a vehicle control. C75 is run at 5 and 10 $\mu\text{g/ml}$

in triplicate as positive controls. After 4 hours of incubation, 0.25 μCi of [^{14}C]acetate (10 μl volume) is added to each well.

After 2 hours of additional incubation, medium is aspirated from the wells and 800 μl of chloroform:methanol (2:1) and 700 μl of 4 mM MgCl_2 is added to each well. Contents of each well are transferred to 1.5 Eppendorf tubes, and spun at full-speed for 2 minutes in a high-speed Eppendorf Microcentrifuge 5415D. After removal of the aqueous (upper) layer, an additional 700 μl of chloroform:methanol (2:1) and 500 μl of 4 mM MgCl_2 are added to each tube and then centrifuged for 1 minutes as above. The aqueous layer is removed with a Pasteur pipette and discarded. An additional 400 μl of chloroform:methanol (2:1) and 200 μl of 4 mM MgCl_2 are added to each tube, then centrifuged and aqueous layer is discarded. The lower (organic) phase is transferred into a scintillation vial and dried at 40 $^{\circ}\text{C}$ under N_2 gas. Once dried, 3 ml of scintillant (APB #NBC5104) is added and vials are counted for ^{14}C . The Beckman Scintillation counter calculates the average cpm values for triplicates.

The IC_{50} for the compounds is defined as the concentration of drug leading to a 50% reduction in [^{14}C]acetate incorporation into lipids compared to controls. This is determined by plotting the average cpm for each inhibitor concentration tested, performing linear regression and computing the best-fit line, r^2 values, and 95% confidence intervals. The average cpm values are computed by the Beckman scintillation counter (Model LS6500) for each compound concentration. Computation of linear regression, best-fit line, r^2 , and 95% confidence intervals are calculated using Prism Version 3.0 (Graph Pad Software).

Carnitine Palmitoyltransferase-1 (CPT-1) Assay

CPT-1 catalyzes the ATP dependent transfer of long-chain fatty acids from acyl-CoA to acyl-carnitine that is inhibited by malonyl-CoA. As CPT-1 requires the mitochondrial

membrane for activity, enzyme activity is measured in permeabilized cells or mitochondria. This assay uses permeabilized cells to measure the transfer of [methyl-¹⁴C]L-carnitine to the organically soluble acyl-carnitine derivative.

MCF-7 cells are plated in DMEM with 10% fetal bovine serum at 10⁶ cells in 24-well plates in triplicate for controls, drugs, and malonyl-CoA. Two hours before commencing the assay, drugs are added at the indicated concentrations made from stock solutions at 10 mg/ml in DMSO, vehicle controls consist of DMSO without drug. Since malonyl-CoA cannot enter intact cells, it is only added in the assay buffer to cells that have not been preincubated with drugs. Following overnight incubation at 37 °C, the medium is removed and replaced with 700 µl of assay buffer consisting of: 50 mM imidazole, 70 mM KCl, 80 mM sucrose, 1 mM EGTA, 2 mM MgCl₂, 1 mM DTT, 1 mM KCN, 1 mM ATP, 0.1% fatty acid free bovine serum albumin, 70 µM palmitoyl-CoA, 0.25 µCi [methyl-¹⁴C]L-carnitine, 40 µg digitonin with drug, DMSO vehicle control, or 20 µM malonyl-CoA. The concentrations of drugs and DMSO in the assay buffer is the same as used in the 2 hr preincubation. After incubation for 6 minutes at 37 °C, the reaction is stopped by the addition of 500 µl of ice-cold 4 M perchloric acid. Cells are then harvested and centrifuged at 13,000 x g for 5 minutes. The pellet is washed with 500 µl ice cold 2mM perchloric acid and centrifuged again. The resulting pellet is resuspended in 800 µl dH₂O and extracted with 150 µl of butanol. The butanol phase is counted by liquid scintillation and represents the acylcarnitine derivative.

Weight Loss Screen for Novel FAS Inhibitors

Balb/C mice (Jackson Labs) are utilized for the initial weight loss screening.

Animals are housed in temperature and 12 hour day/night cycle rooms and fed mouse chow and water *ad lib*. Three mice are utilized for each compound tested with vehicle controls in triplicate
5 per experiment. For the experiments, mice are housed separately for each compound tested three mice to a cage. Compounds are diluted in DMSO at 10 mg/ml and mice are injected intraperitoneally with 60 mg/kg in approximately 100 µl of DMSO or with vehicle alone. Mice are observed and weighed daily; average weights and standard errors are computed with Excel (Microsoft). The experiment continues until treated animals reach their pretreatment weights.

10 Select compounds are tested in animals housed in metabolic cages. Dosing of animals are identical to the screening experiments with three animals to a single metabolic cage. Animal weights, water and food consumption, and urine and feces production are measured daily. The results for the testing of Compounds 21 and 44 are shown in FIG. 10.

Antimicrobial Properties

A broth microdilution assay is used to assess the antimicrobial activity of the compounds. Compounds are tested at twofold serial dilutions, and the concentration that inhibits visible growth (OD₆₀₀ at 10% of control) is defined as the MIC. Microorganisms tested include
15 *Staphylococcus aureus* (ATCC # 29213), *Enterococcus faecalis* (ATCC # 29212), *Pseudomonas aeruginosa* (ATCC # 27853), and *Escherichia coli* (ATCC # 25922). The assay is performed in
20 two growth media, Mueller Hinton Broth and Trypticase Soy Broth.

A blood (Tsoy/5% sheep blood) agar plate is inoculated from frozen stocks maintained in T soy broth containing 10% glycerol and incubated overnight at 37° C. Colonies are suspended in

sterile broth so that the turbidity matches the turbidity of a 0.5 McFarland standard. The inoculum is diluted 1:10 in sterile broth (Mueller Hinton or Trypticase soy) and 195 μ l is dispensed per well of a 96-well plate. The compounds to be tested, dissolved in DMSO, are added to the wells in 5 μ l volume at the following concentrations: 25, 12.5, 6.25, 3.125, 1.56 and 0.78 μ g/ml in duplicate. Additional concentrations are tested if required. 5 μ l of DMSO added to duplicate wells are the vehicle control. Serial dilutions of positive control compounds, vancomycin (*E. faecalis* and *S. aureus*) and tobramycin (*E. coli* and *P. aeruginosa*), are included in each run.

After 24 hours of incubation at 37 °C, plates are read at OD₆₀₀ on a Molecular Devices SpectraMax Plus Spectrophotometer. Average OD₆₀₀ values are computed using SOFTmax Pro Software (Molecular Devices) and MIC values are determined by linear regression analysis using Prism version 3.02 (Graph Pad Software, San Diego). The MIC is defined as the concentration of compound required to produce an OD₆₀₀ reading equivalent to 10% of the vehicle control reading.

15 *In Vivo Testing for Anti-Tumor Activity*

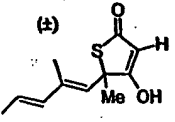
The results of this experiment are shown in FIG. 11. Subcutaneous flank xenografts of the human colon cancer cell line, HCT-116 in nu/nu female mice (Harlan) were used to study the anti-tumor effects of Compound 36 *in vivo*. All animal experiments complied with institutional animal care guidelines. 10⁷ HCT-116 cells (~0.1 ml packed cells) were xenografted from culture in DMEM supplemented with 10% FBS into 10 athymic mice. Treatment began when measurable tumors developed about 4 days after inoculation. Compound 36 (10 mg/kg) was diluted into 20 μ l DMSO and treated intraperitoneally, i.p. Five animals

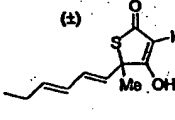
received JMM-II-265 i.p. at days indicated by arrows in FIG. 11, and 5 received DMSO control.

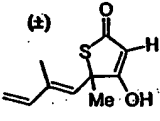
Tumors were measured on days indicated. Error bars represent standard error of the mean.

Results of the biological testing

5

 17	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	71.2 ug/ml	17.3 ug/ml	>80 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg : 4.1% (day1)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	52 ug/ml	87 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Neg	Neg	Neg	Neg

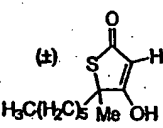
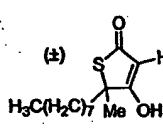
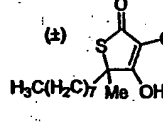
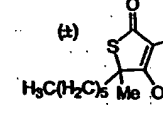
 19	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	50.0 ug/ml	16.9 ug/ml	>80 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg : 3.2% (day5)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	202 ug/ml	85 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	186 ug/ml	Neg	225 ug/ml	Neg

<div></div> <div>21</div>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	8.8 + 0.02ug/ml	40.3 + 11.5ug/ml	>80 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	95% of control	60 mg/kg : 7.8% (day3)		
	at 20ug/ml (MCF7)			
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	235 ug/ml	102 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	220 ug/ml	Neg	290 ug/ml	Neg

(4)

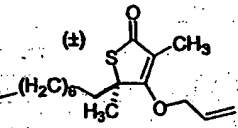
18

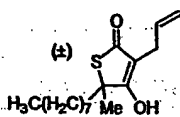
FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
8.6 ± 1.7 ug/ml	25.7 ug/ml	59.4 ± 6.4 ug/ml	43.9 ± 4.8 ug/ml
CPT I Stim	Weight Loss		
115% of control	60 mg/kg : 11% (day6)		
at 20ug/ml (MCF7)			
SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
Neg	55 ug/ml	Neg	Neg
EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
78 ug/ml	42 ug/ml	Neg	263 ug/ml

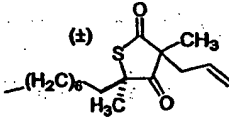
 <p>33</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	16.5 ± 3.8 ug/ml	>80 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 3 of 3 dead (day 4)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	48 ug/ml	31 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	98 ug/ml	43 ug/ml	Neg	Neg
 <p>32</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	4.5 ug/ml	12.6 ± 4.4 ug/ml	17.6 ± 0.1 ug/ml	28.7 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 2% and 0.3% (day1), 30mg/kg: 4.8% (day3)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Neg	47 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	16.9 ug/ml	3.3 ug/ml	Neg	278 ug/ml
 <p>34</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	49.2 ± 1.9 ug/ml	16.5 ± 5.7 ug/ml	48.0 ± 1.4 ug/ml	29.4 ± 4.3 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 0% (day1), 30mg/kg: +1% (day1)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	45 ± 2 ug/ml	23.5 ± 0.4 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	44 ug/ml	105 ug/ml	Neg	290 ug/ml
 <p>36</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	14.0 ± 2.8 ug/ml	9.4 ± 1.5 ug/ml	26.3 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 3 of 3 dead (day1); 30mg/kg: 8.7% (day1) 10 mg/kg (multiple doses): 1% (day3)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	45 ug/ml	48 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	43 ug/ml	126 ug/ml	Neg	264 ug/ml

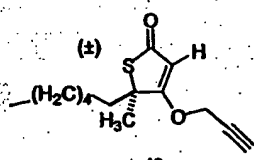
5

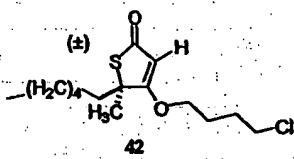
10

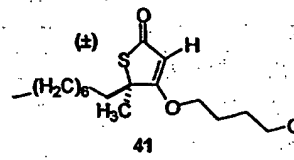
 47	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Not Tested	Neg	Not Tested	15.1 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

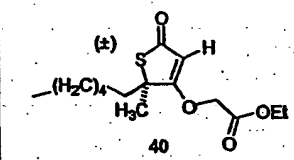
 44	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	23.1 ± 17.4ug/ml	55.0 ± 2.0 ug/ml	22.3 ug/ml
	CPT I Stim	Weight Loss		
	125% of control	60 mg/kg: 7.9% and 8.0% (day1)		
	at 20ug/ml (MCF7)			
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Neg	98 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Neg	169 ug/ml	Neg	Neg

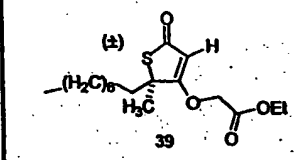
 48	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	14.9 ug/ml	50.4 ± 4.7 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Neg	97 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	133 ug/ml	91 ug/ml	Neg	Neg

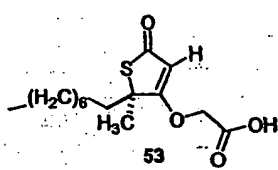
 49	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	21.9 ± 14.4 ug/ml	8.9 ± 2.3 ug/ml	12.1 ± 1.5 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 3 of 3 dead(day2); 30mg/kg: 5.9% (day 3)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	53 ug/ml	80 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Neg	83 ug/ml	203 ug/ml	Neg

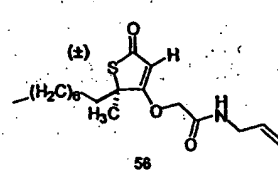
 42	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	8.6 ug/ml	20.8 ± 0.9 ug/ml	16.3 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	164 ug/ml	50 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	117 ug/ml	165 ug/ml	Neg	Neg

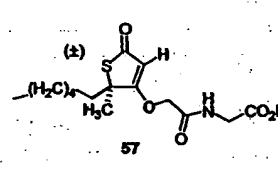
 41	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	6.8 ug/ml	35.3 ± 2.2 ug/ml	10.3 ± 0.3 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	115 ug/ml	134 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	64 ug/ml	Neg	Neg	Neg

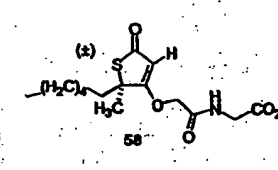
 40	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	14.2 ± 0.2 ug/ml	39.6 ± 2.2 ug/ml	17.0 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Neg	129 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	21.3 ug/ml	Neg	Neg	281 ug/ml

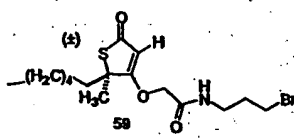
 39	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	10.8 ± 5.8 ug/ml	35.3 ± 10.4 ug/ml	17.9 ± 5.1 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 1.8% and 3.6% (day 1); 30 mg/kg: 4.5% (day 1)		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Neg	83 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	86 ug/ml	Neg	Neg	Neg

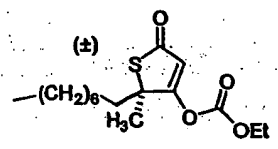
 53	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	13.8 ± 1.1 ug/ml	50.3 ± 2.8 ug/ml	33.7 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	98 ug/ml	60 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	77 ug/ml	164 ug/ml	Neg	Neg

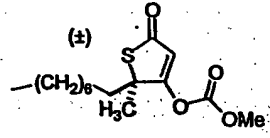
 56	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	Neg	12.1 ± 0.6 ug/ml	10.4 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg; 1.8% (day2); 15 mg/kg; 0% (day1)		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	28 ug/ml	31 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

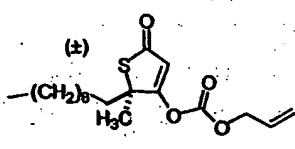
 57	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	9.8 ug/ml	40.5 ± 5.1 ug/ml	32.5 ± 11.7 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	69 ug/ml	111 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Neg	Neg	156 ug/ml	Neg

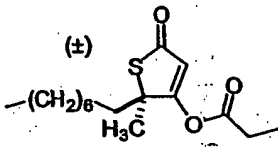
 58	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	6.6 ug/ml	>80 ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg; 3.5% (day 2)		
	SA/MH (MIC)	SA/Tsoy (MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	78 ug/ml	225 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy (MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	121 ug/ml	173 ug/ml	Neg	235 ug/ml

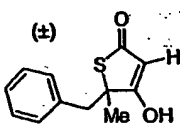
 59	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	6.7 ug/ml	21.2 ± 1.1 ug/ml	12.6 ± 3.7 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	147 ug/ml	237 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	99 ug/ml	121 ug/ml	Neg	293 ug/ml

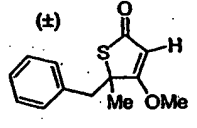
 61	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	3.0 ± 0.8	14.5 ± 6.9 ug/ml	15.1 ± 2.6 ug/ml	31.4 ± 5.7 ug/ml
	CPT I Stim	Weight Loss		
	150% of control at 20ug/ml (MCF7)	60 mg/kg: 6.9% and 5.7% (day2); 30 mg/kg: 1.3% (day4)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	45 ug/ml	69 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	97 ug/ml	62 ug/ml	Neg	Neg

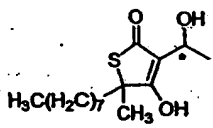
 62	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	1.8 ug/ml	10.7 ug/ml	21.6 ± 0.2 ug/ml	41.1 ± 14.1 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 7.65% (day 1)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	64 ug/ml	41 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	73 ug/ml	65 ug/ml	296 ug/ml	Neg

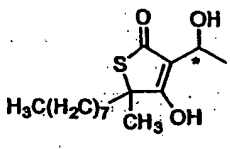
 63	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	5.5 ug/ml	14.2 ug/ml	34.9 ± 10.0 ug/ml	35.8 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 6.2% (day2) ; 30 mg/kg: 1% (day 2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	57 ug/ml	28 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	79 ug/ml	75 ug/ml	82 ug/ml	87 ug/ml

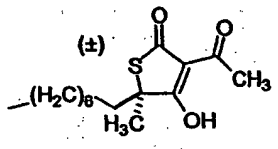
	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	22.6 ug/ml	26.8 ± 0.6 ug/ml	38.6 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	88 ug/ml	62 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	102 ug/ml	147 ug/ml	Neg	Neg

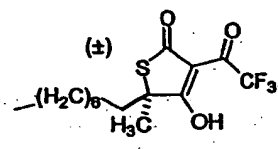
	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	Neg	>80. ug/ml	>50 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 1.6% (day 2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	57 ug/ml	67 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	128 ug/ml	Neg	Neg	299 ug/ml

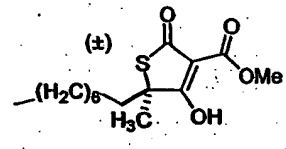
	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	Not Tested	61.3 ± 3.9 ug/ml	20.9 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	83 ug/ml	129 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	170 ug/ml	189 ug/ml	Neg	Neg

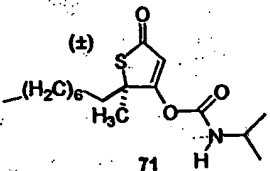
	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	2.8 ug/ml	21.7 ug/ml	21.0 ± 2.9 ug/ml	23.2 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	30 mg/kg: 0.2% (day2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	53.0 ± 2.1 ug/ml	16.2 ± 3.8 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	77 ug/ml	25 ug/ml	Neg	249 ug/ml

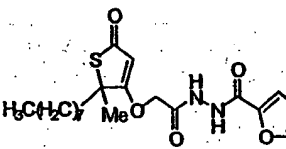
 <p>67</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	3.3 ug/ml	17.6 ug/ml	23.9 ± 2.9 ug/ml	19.5 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	53.1 ± 0.5 ug/ml	12.0 ± 0.5 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	66 ug/ml	21 ug/ml	Neg	Neg

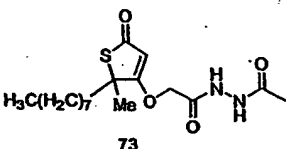
 <p>68</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	12.1 ± 0.1 ug/ml	12.5 ± 0.7 ug/ml	8.4 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 8.2% (day2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	3.4 ug/ml	1.4 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	2.5 ug/ml	2.0 ug/ml	Neg	177 ug/ml

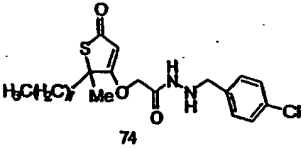
 <p>69</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	41.0	14.7 ug/ml	18.4 ± 2.7 ug/ml	45.3 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	54 ug/ml	65 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

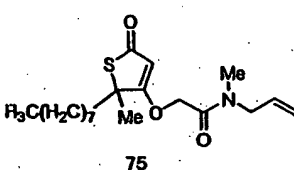
 <p>70</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Not Tested	Not Tested	Not Tested	Not Tested
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Not Tested	23 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

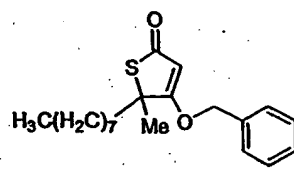
 71	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	80.6 ug/ml	23.1+ 13.2 ug/ml	45.7 + 25.9ug/ml	22.5 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	Not Tested		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	32 ug/ml	39 ug/ml	Neg	Neg
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	60 ug/ml	64 ug/ml	Neg	Neg

 72	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	16.4 ug/ml	26.4 + ug/ml (M)	26.3 ug/ml
			21.3 ug/ml (OV)	
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 6.3% (day 4)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

 73	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	11.5 ug/ml	25.3 + ug/ml (M)	28.7 ug/ml
			16.0 ug/ml (OV)	
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 5.9% (day 4)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

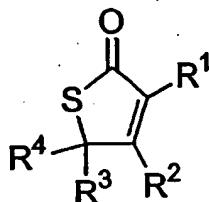
 74	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	10.5 + 2.6 ug/ml	6.7 + ug/ml (M)	<5 ug/ml
			8.8 ug/ml (OV)	
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg: 1.4% (day 2)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAE/MH (MIC)	PSAE/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

 <p>75</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	25.7 ug/ml	9.2 ± 2.2 ug/ml (M)	9.0 ug/ml
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg; 1.5% (day 3)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAB/MH (MIC)	PSAB/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

 <p>76</p>	FAS (IC ₅₀)	¹⁴ C (IC ₅₀)	XTT (IC ₅₀)	Cr. Violet (IC ₅₀)
	Neg	Neg (stim)	29.2 ± ug/ml (M)	9.5 ug/ml
			27.8 ug/ml (OV)	
	CPT I Stim	Weight Loss		
	Not Tested	60 mg/kg; 5.2% (day 3)		
	SA/MH (MIC)	SA/Tsoy(MIC)	PSAB/MH (MIC)	PSAB/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested
	EF/MH (MIC)	EF/Tsoy(MIC)	Ecoli/MH (MIC)	Ecoli/Tsoy (MIC)
	Not Tested	Not Tested	Not Tested	Not Tested

We claim:

-1. A compound of formula:



I

wherein:

$R^1 = H$

$R^2 = -OH, -OR^5, -OCH_2C(O)R^5, -OCH_2C(O)NHR^5, -OC(O)R^5, -OC(O)OR^5, -OC(O)NHNH-R^5,$
or $-OC(O)NR^5R^6$, where R^5 is H, C_1 - C_{20} alkyl, cycloalkyl, alkenyl, alkynyl, aryl,

10 arylalkyl, or alkylaryl, and where R^5 can optionally contain halogen atoms;

R^3 and R^4 , the same or different from each other, are C_1 - C_{20} alkyl, cycloalkyl, alkenyl, aryl,
arylalkyl, or alkylaryl;

with the proviso that when R^2 is $-OH, -OCH_3$, or $-OC(O)CF_3$ and R_3 is $-CH_3$, then R_4 is not $-CH_2CH_2OH, -CH_2-(C_6H_5)$, or $-CH=CH-CH_3$, and

15 and the further proviso that when R^3 is $-CH_2-(C_6H_5)$, then R^4 is not $-CH_3$ or $-CH_2CH_3$.

2. A compound according to claim 1, wherein R^5 is H, C_1 - C_{10} alkyl, cycloalkyl,
alkenyl, aryl, arylalkyl, or alkylaryl.

3. A compound according to claim 2, wherein R^5 is H, or C_1 - C_{10} alkyl.

4. A compound according to claim 1, wherein R^3 and R^4 are each independently H,

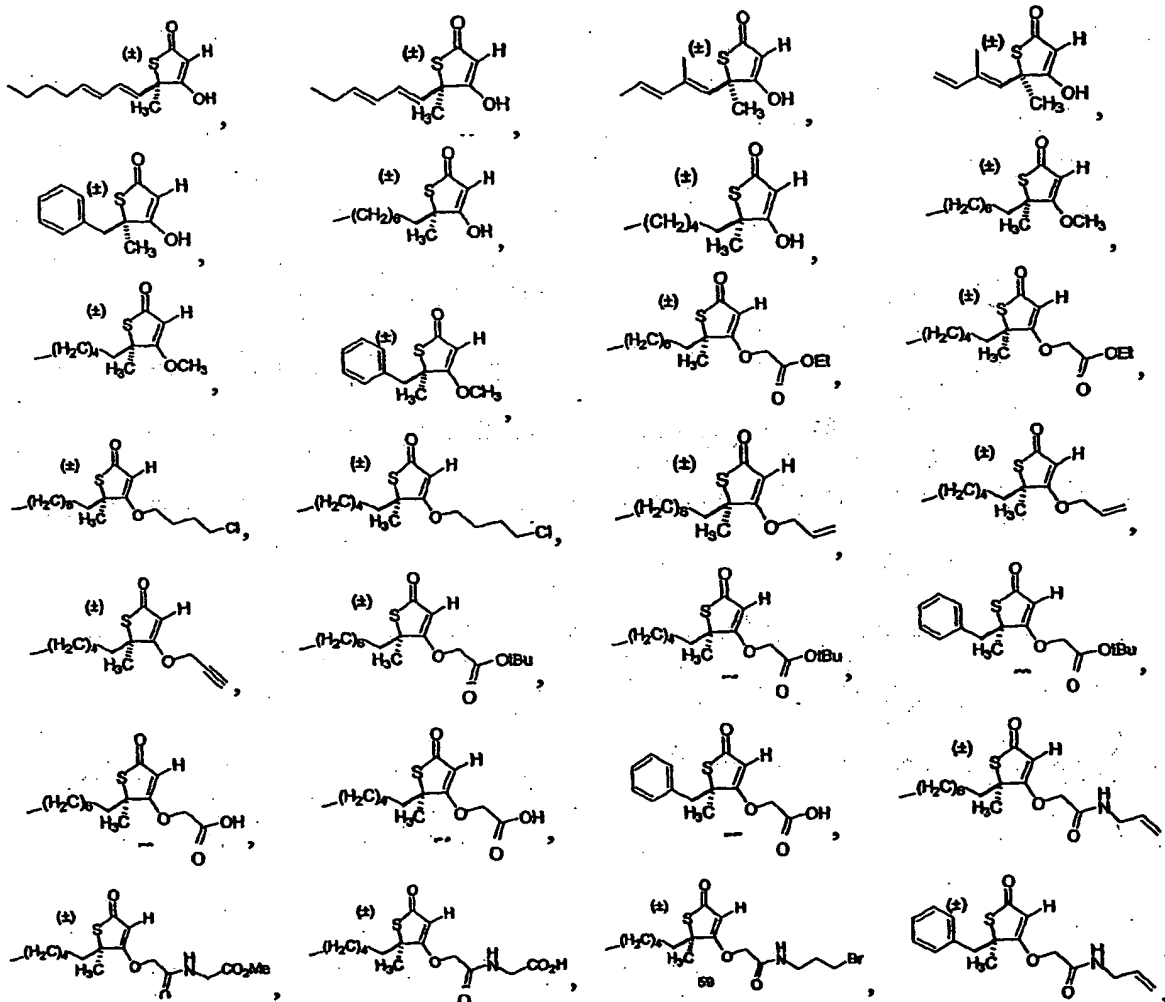
20 C_1 - C_{10} alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

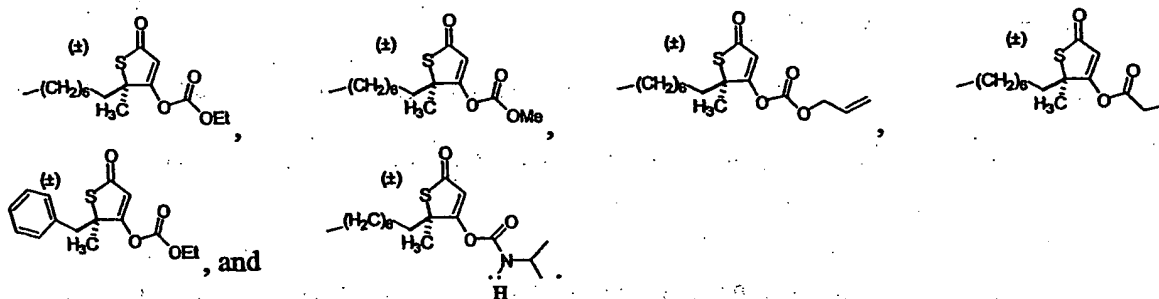
5. A compound according to claim 4, wherein R³ and R⁴ are each independently H, or C₁-C₁₀ alkyl.

6. A compound according to claim 1, wherein, R^3 is $-H$ or $-CH_3$.

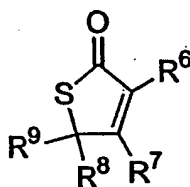
7. A compound according to claim 1, wherein R^4 is $-nC_6-C_8$ alkyl.

- 5 8. A compound according to claim 1, wherein the compound is selected from the group consisting of





9. A compound of formula II:



II

wherein

$R^6 = C_2-C_{20}$ alkyl, cycloalkyl, alkenyl, alkynyl, aryl, arylalkyl, or alkylaryl, $-CHR^{10}OR^{11}$,

$-CO(O)R^{10}$, $-C(O)NR^{10}R^{11}$, $-CH_2C(O)R^{10}$, or $-CH_2C(O)NHR^{10}$, where R^{10} and R^{11} are
 10 each independently H, C_1-C_{10} alkyl, cycloalkyl, alkenyl, alkynyl, aryl, arylalkyl, or
 alkylaryl, optionally containing halogen atoms, but R^6 is not di-, tri-, or tetra-alkyl
 substituted phenyl,

$R^7 = -OH$, $-OR^{12}$, $-OCH_2C(O)R^{12}$, $-OCH_2C(O)NHR^{12}$, $-OC(O)R^{12}$, $-OC(O)OR^{12}$, $OC(O)NHNH-R$
 or $-OC(O)NR^{12}R^{13}$, where R^{12} and R^{13} are each independently H, C_1-C_{20} alkyl, cycloalkyl,
 15 alkenyl, aryl, arylalkyl, or alkylaryl, and where R^{12} and R^{13} can optionally contain halogen
 atoms;

R^8 and R^9 , the same or different from each other, are C_1-C_{20} alkyl, cycloalkyl, alkenyl, aryl,
 arylalkyl, or alkylaryl,

with the following provisos:

when R⁶ is ethyl, if R⁸ and R⁹ are not the same, then R⁸ or R⁹ are not ethyl, -CH₂COOH, -

CH₂C(O)NH₂, -CH₂-(C₆H₅), but R⁸ and R⁹ can be the same, even if R⁶ is ethyl,

and

5 when R⁶ is phenyl, and R⁷ is -OH, R⁸ and R⁹ cannot simultaneously be -CH₃ and

-propenyl, and

when R⁶ is phenyl, R⁸ and R⁹ cannot simultaneously be -CH₃ or -CH₂-(C₆H₅).

10. A compound according to claim 9, wherein R¹⁰ is C₁-C₁₀ alkyl, cycloalkyl,

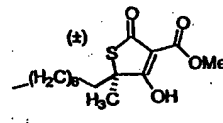
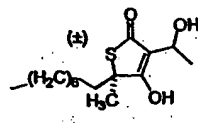
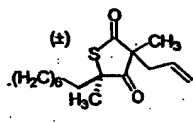
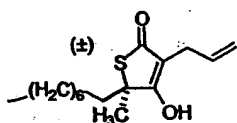
10 alkenyl, aryl, arylalkyl, or alkylaryl.

11. A compound according to claim 9, wherein R⁸ is -H or -CH₃.

12. A compound according to claim 9, wherein R⁹ is -nC₆-C₈ alkyl.

13. A compound according to claim 9, wherein the compound is selected from the

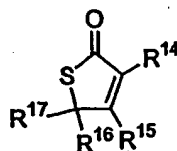
group consisting of:



, and

15

14. A compound of formula III:



III

wherein

$R^{14} = -C(O)R^{18}$, where R^{18} is H, C₁-C₁₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl,

optionally containing halogen atoms.

$R^{15} = -OH, -OR^{19}, -OCH_2C(O)R^{19}, -OCH_2C(O)NHR^{19}, -OC(O)R^{19}, -OC(O)OR^{19},$

$-OC(O)NHNH-R^{19}$, or $-OC(O)NR^{19}R^{20}$, where R^{19} and R^{20} are each independently H, C₁-

C₂₀ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, and where R^{19} and R^{20} can each

optionally contain halogen atoms;

R^{16} and R^{17} , the same or different from each other, are C₁-C₂₀ alkyl, cycloalkyl, alkenyl, aryl,

arylalkyl, or alkylaryl,

with the following provisos:

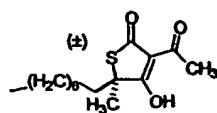
- when R^{14} is $-C(O)CH_3$, and R^{16} and R^{17} are not identical, then either R^{16} or R^{17} are not

are not geranyl, p-fluorobenzyl, cinnamyl, farnesyl, methyl, or $-CH_2-(C_6H_5)$, and

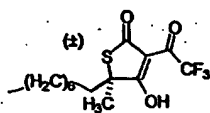
- when R^{14} is $-C(O)C_6H_5$, then either R^{16} or R^{17} are not are not methyl.

15. A compound according to claim 14, wherein the compound is selected from the

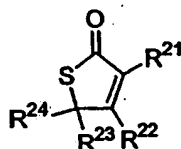
group consisting of:



, and



16. A pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula IV:



IV

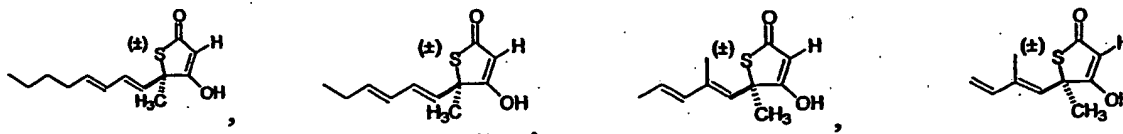
wherein:

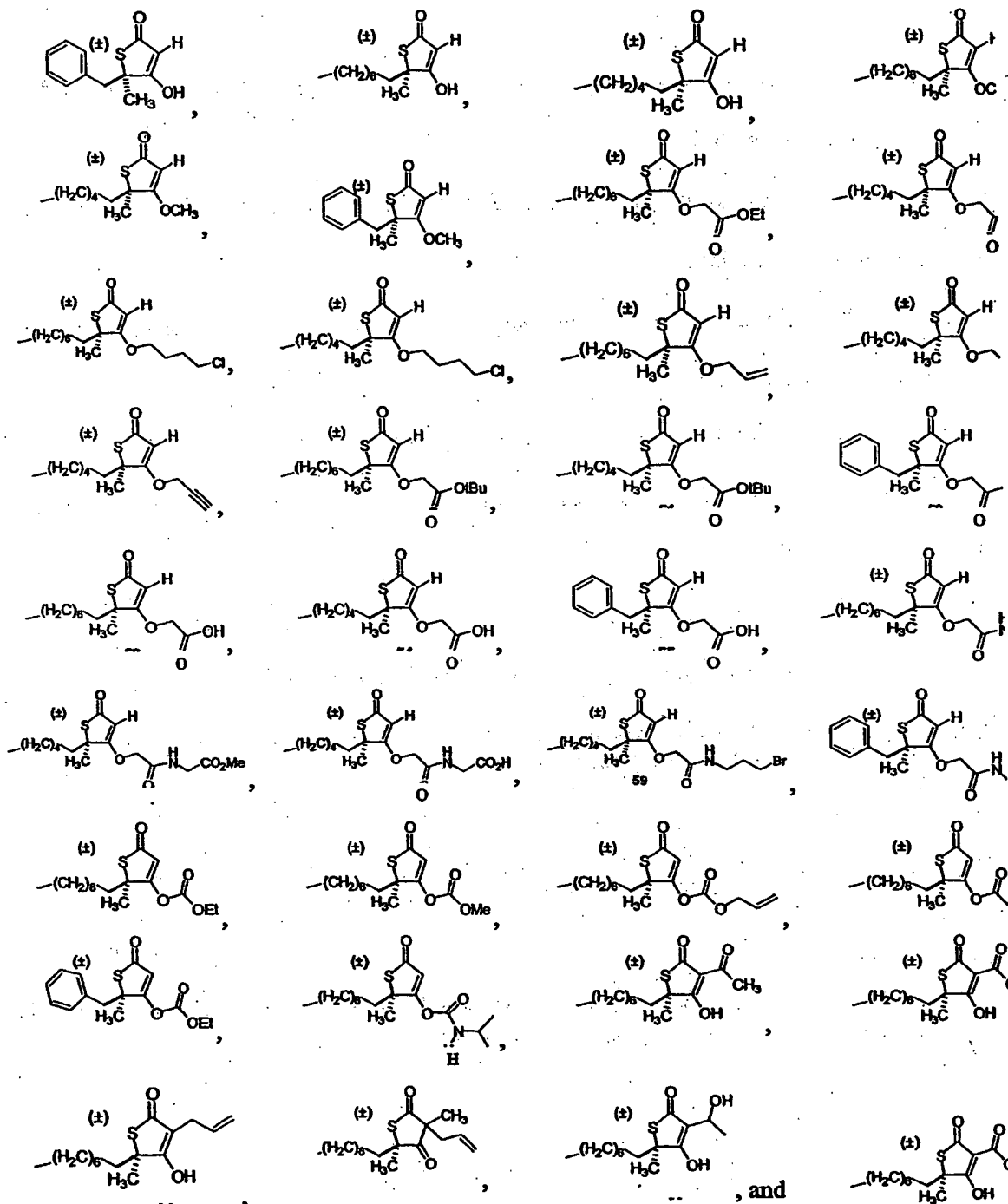
10 $R^{21} = \text{H, C}_1\text{-C}_{20} \text{ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, } -\text{CH}_2\text{OR}^{25}, -\text{C(O)R}^{25},$
 $-\text{CO(O)R}^{25}, -\text{C(O)NR}^{25}\text{R}^{26}, -\text{CH}_2\text{C(O)R}^{25}, \text{ or } -\text{CH}_2\text{C(O)NHR}^{25},$ where R^{25} and R^{26} are
 each independently H, $\text{C}_1\text{-C}_{10}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl,
 optionally containing one or more halogen atoms.

$R^{22} = -\text{OH, } -\text{OR}^{27}, -\text{OCH}_2\text{C(O)R}^{27}, -\text{OCH}_2\text{C(O)NHR}^{27}, -\text{OC(O)R}^{27}, -\text{OC(O)OR}^{27},$
 15 $\text{OC(O)NHNH-R}^{27}, \text{ or } -\text{OC(O)NR}^{27}\text{R}^{28},$ where R^{27} and R^{28} are each independently H, $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl, and where R^{27} and R^{28} can each
 optionally contain halogen atoms;

R^{23} and R^{24} , the same or different from each other, are $\text{C}_1\text{-C}_{20}$ alkyl, cycloalkyl, alkenyl, aryl, arylalkyl, or alkylaryl.

20 17. A pharmaceutical composition according to claim 16, wherein the compound is selected from the group consisting of:





18. A pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula I.

19. A pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula II.

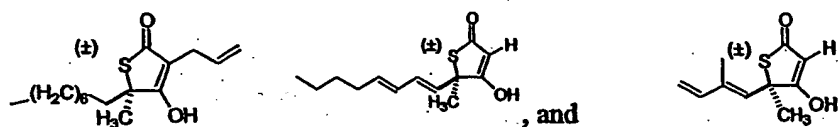
20. A pharmaceutical composition comprising a pharmaceutical diluent and a compound of formula III.

21. A method of inducing weight loss in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 16 to said subject.

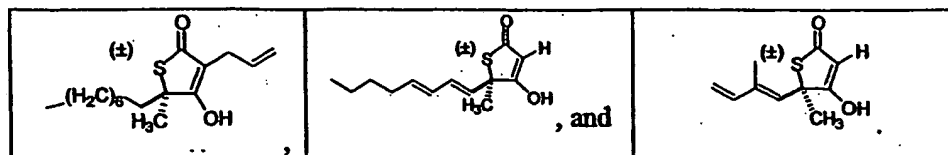
22. The method of claim 21, wherein the subject is a human.

23. The method of claim 21, wherein the subject is an animal.

24. The method of claim 22, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:



25. The method of claim 23, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:

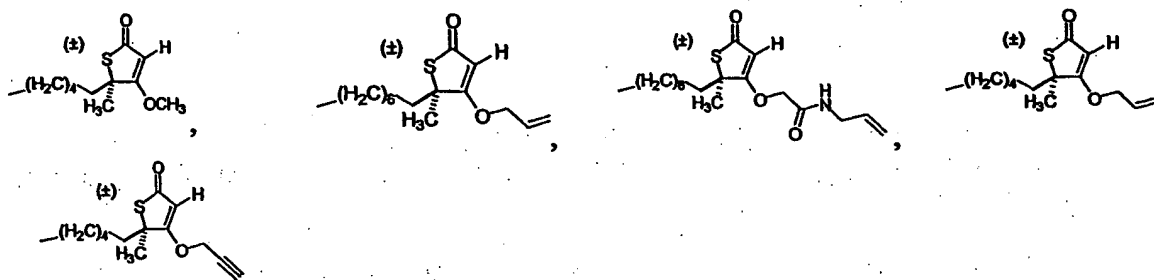


26. A method of treating cancer in an animal or human subject, comprising administering an effective amount of a pharmaceutical composition according to claim 16 to said subject.

5 27. The method of claim 26, wherein the subject is a human.

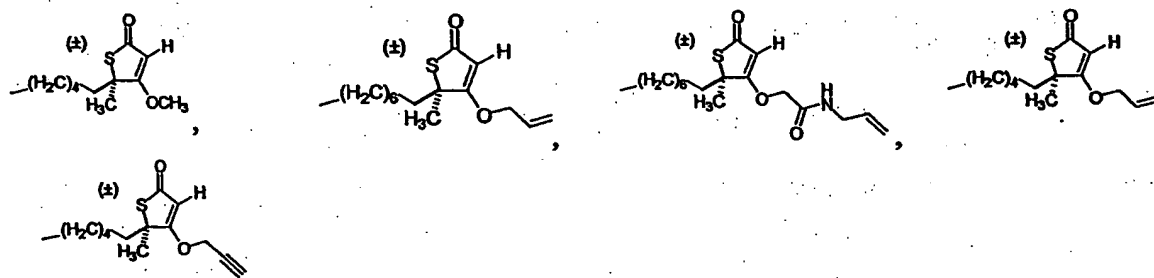
28. The method of claim 26, wherein the subject is an animal.

10 29. The method of claim 27, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:



30. The method of claim 28, wherein the pharmaceutical composition comprises a compound selected from the group consisting of:

15



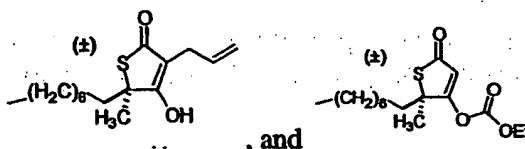
31. A method of stimulating the activity of CPT-1 in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 16 to said subject.

20

32. The method of claim 31, wherein the subject is a human.

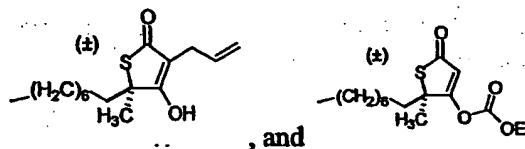
33. The method of claim 31, wherein the subject is an animal.

25 34. The method of claim 32, wherein the compound is selected from the group consisting of:



35. The method of claim 33, wherein the compound is selected from the group consisting of:

5



10 36. A method of inhibiting the activity of neuropeptide-Y in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 16 to said subject.

37. The method of claim 36, wherein the subject is a human.

15

38. The method of claim 36, wherein the subject is an animal.

39. A method of inhibiting fatty acid synthase activity in an animal or human subject comprising administering an effective amount of a pharmaceutical composition according to claim 16 to said subject.

20

40. The method of claim 16, wherein the subject is a human.

41. The method of claim 16, wherein the subject is an animal.

25 42. A method of inhibiting growth of invasive microbial cells in an animal or human subject comprising the administration of an effective amount of a pharmaceutical composition according to claim 16 to said subject.

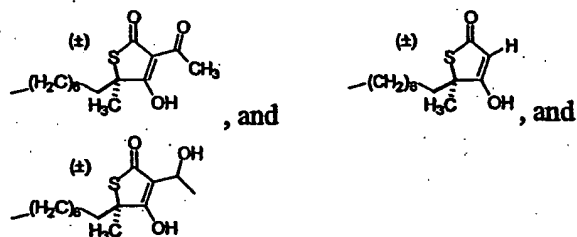
43. The method of claim 42, wherein the subject is a human.

30

44. The method of claim 42, wherein the subject is an animal.

35

45. The method of claim 43, wherein the compound is selected from the group consisting of:



5 46. The method of claim 44, wherein the compound is selected from the group consisting of:

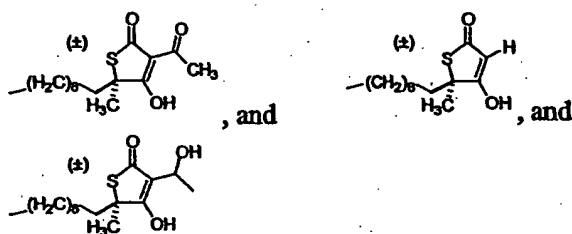


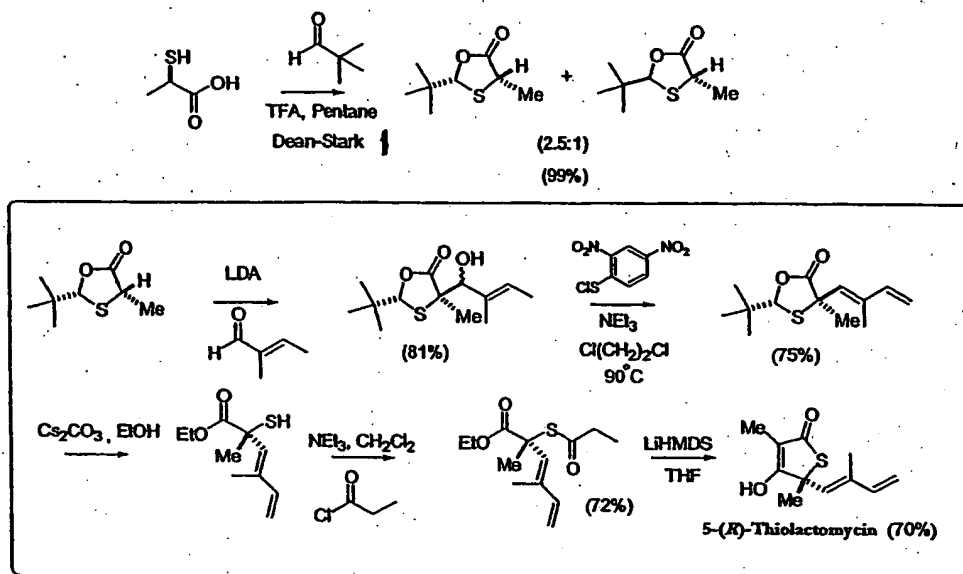
FIG. 1**Scheme 1. Asymmetric Synthesis of 5-(*R*)-Thiolactomycin.**

FIG. 2

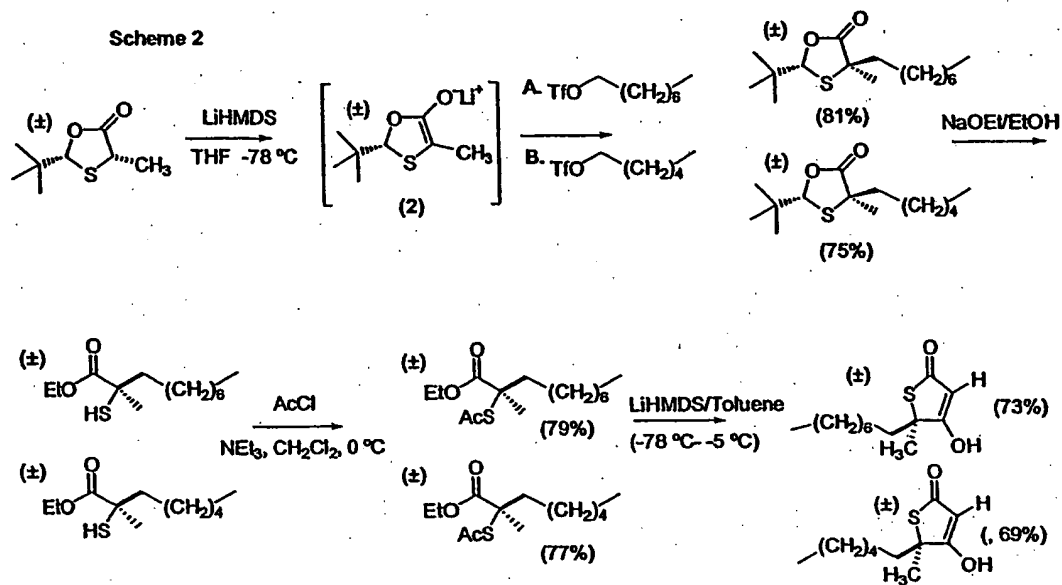


FIG. 3

Scheme 3.

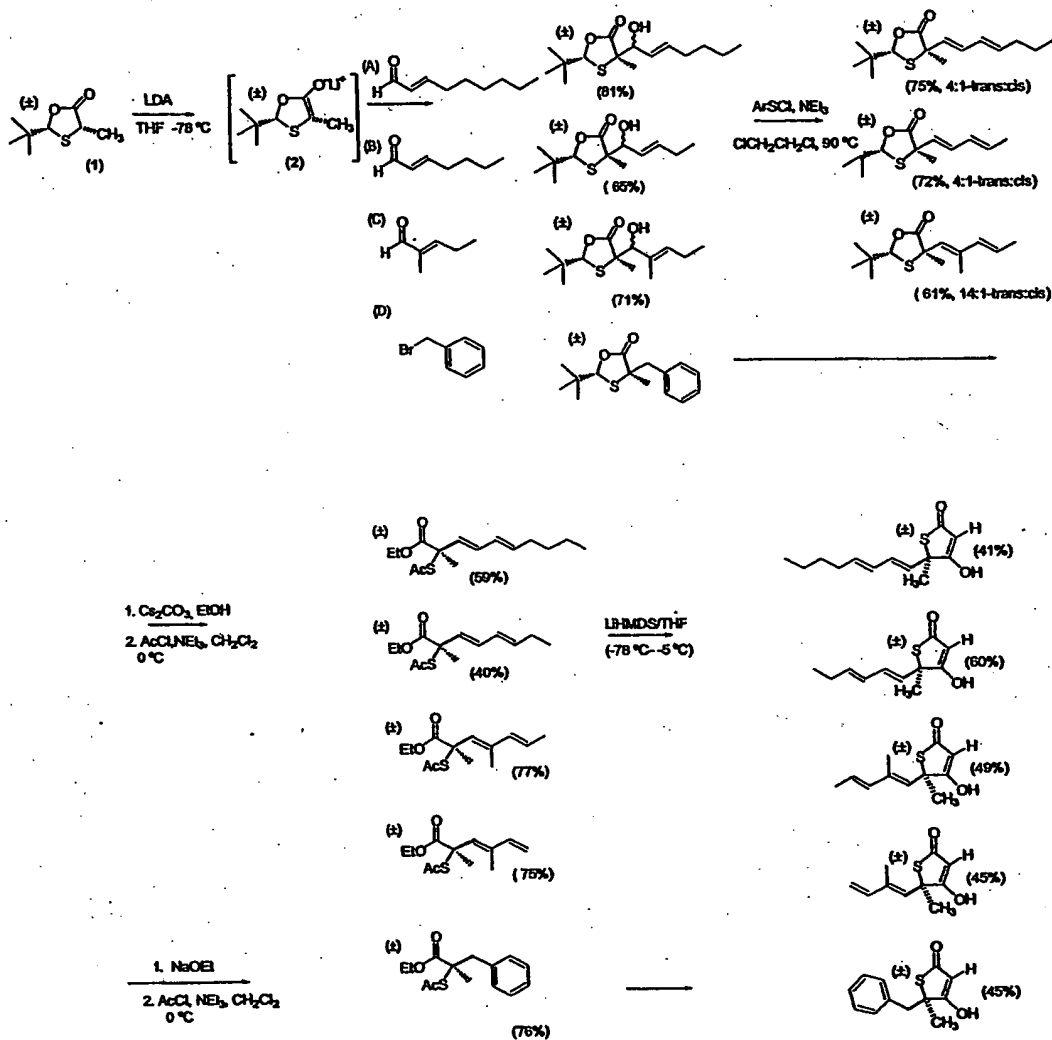


FIG. 4

Scheme 4.

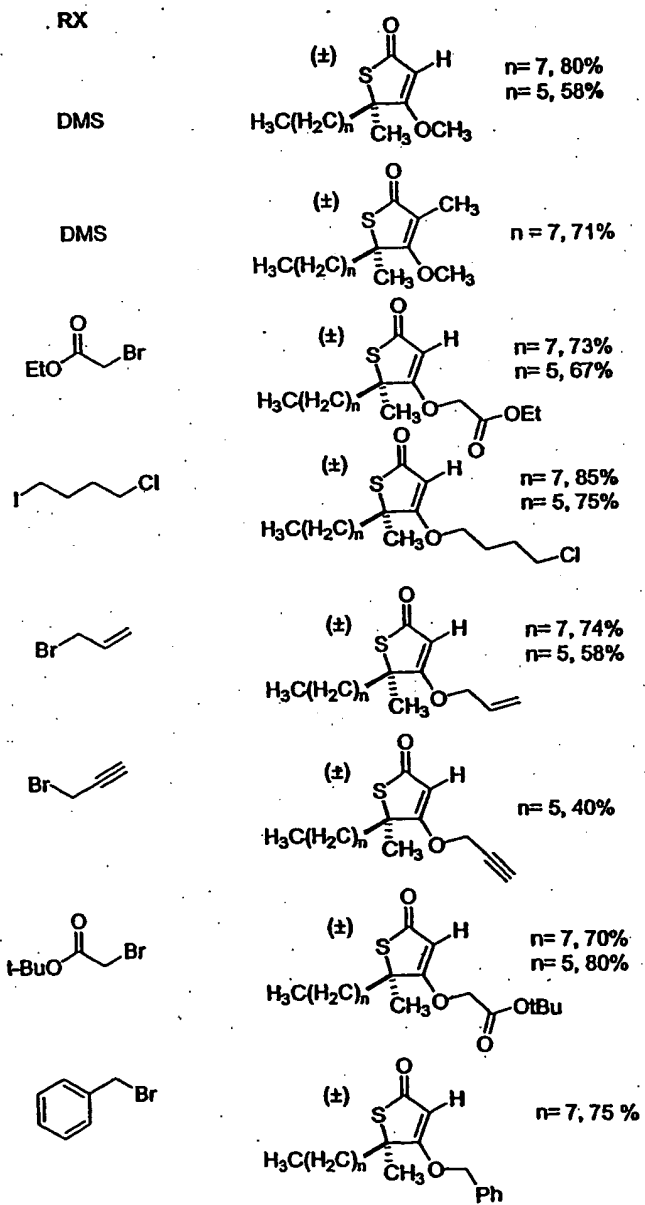
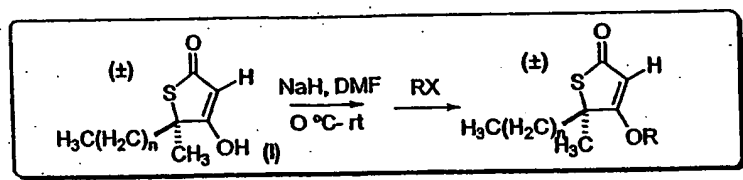


FIG. 5

Scheme 5.

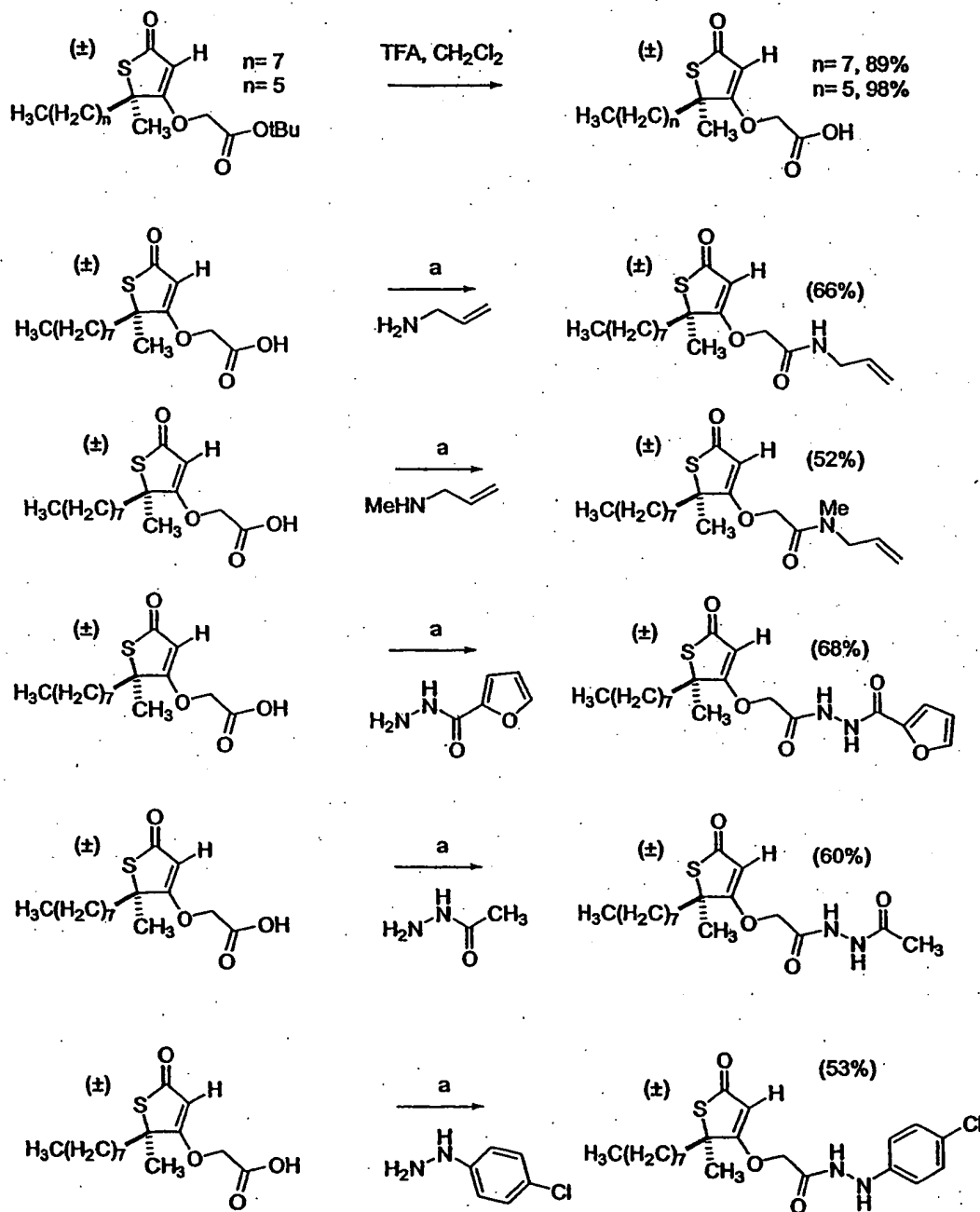


FIG. 6

Scheme 6.

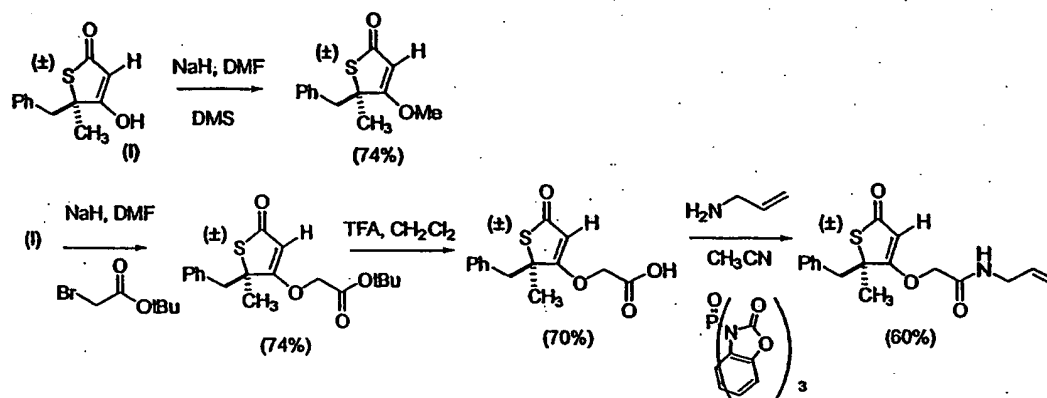


FIG. 7

Scheme 7.

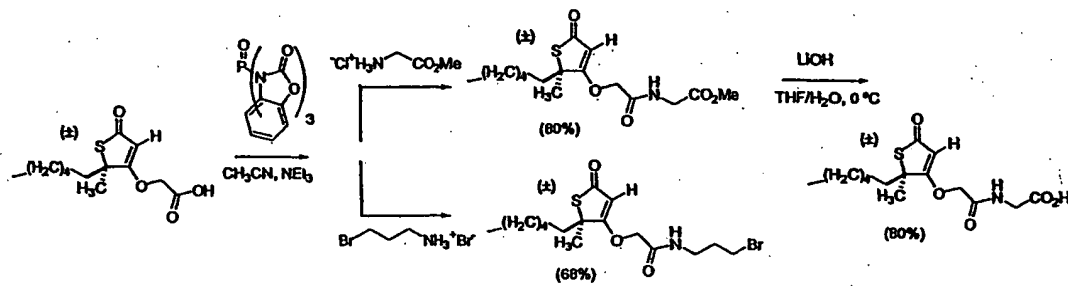


FIG. 8

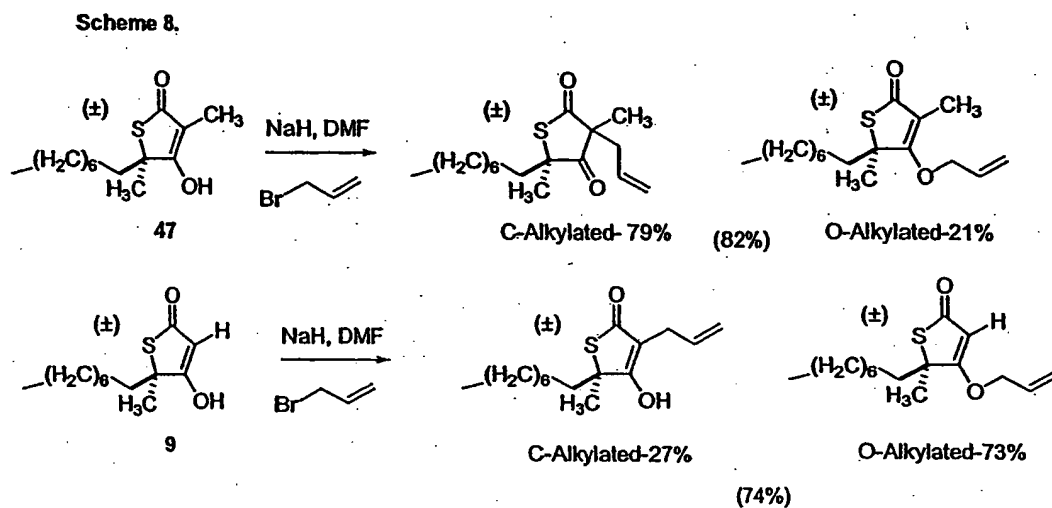


FIG. 9

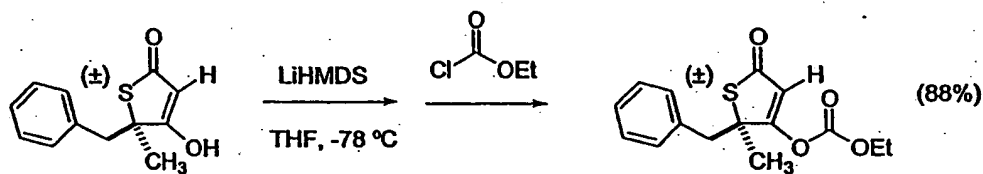
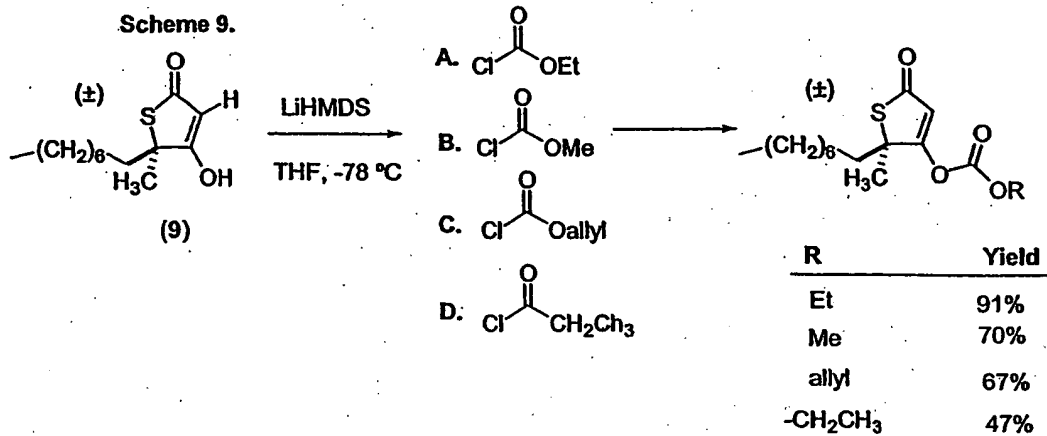


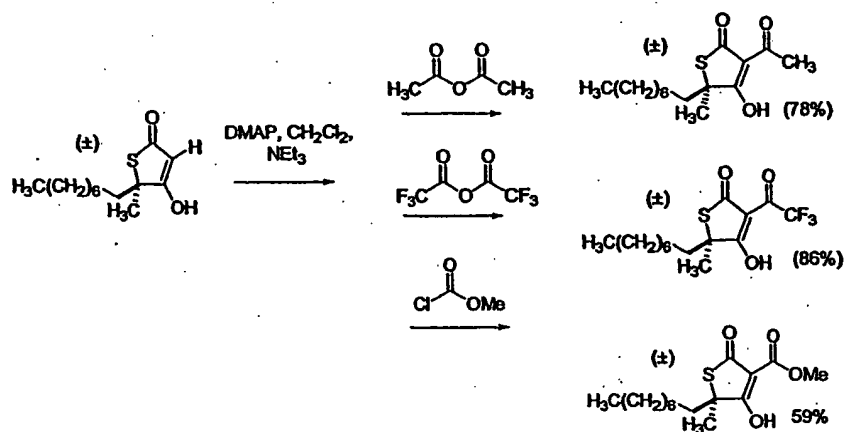
FIG. 10**Scheme 10.**

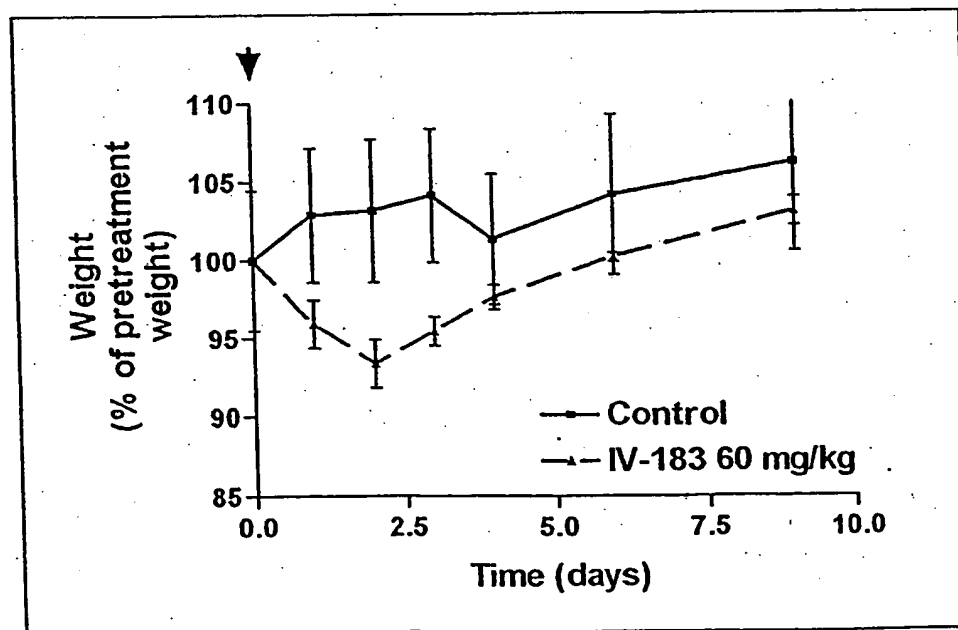
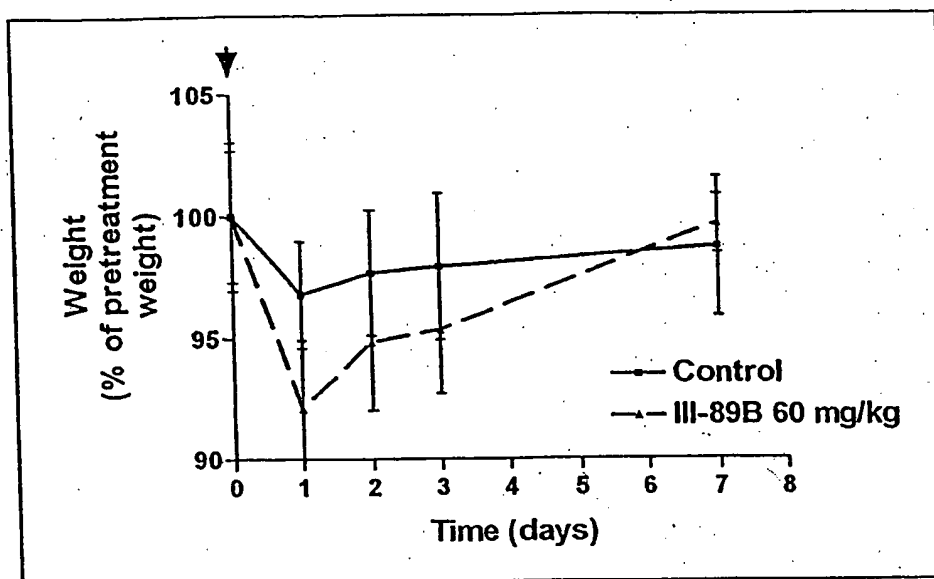
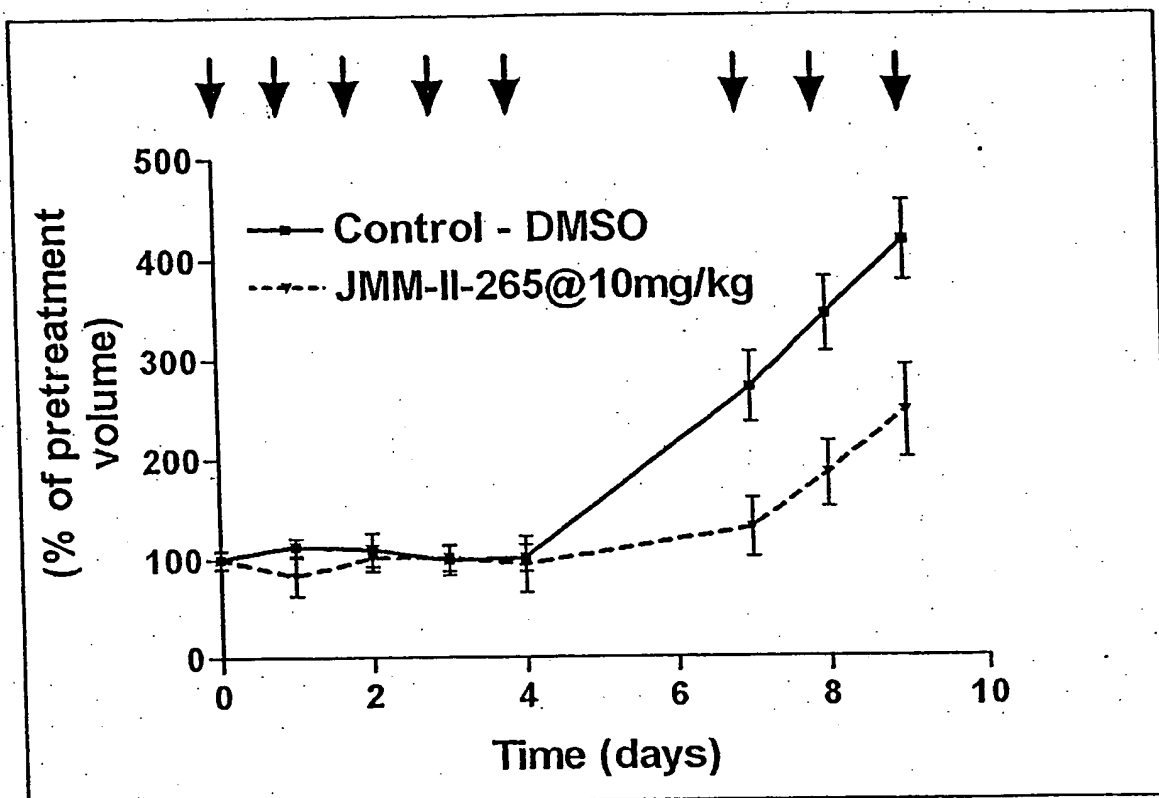
FIG. 11

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/21700

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 333/32; A61K 31/38

US CL : 549/62, 65, 66; 514/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 549/62, 65, 66; 514/445

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,391,912 B1 (HAGEMANN et al.) 21 May 2002 (21.05.2002), abstract.	1-46
A	US 6,380,214 B1 (GANT et al.) 30 April 2002 (30.04.2002), columns 2-4.	1-46
A	US 6,376,682 B1 (YAMAHARA) 23 April 2002 (23.04.2002), abstract.	1-46

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 November 2003 (17.11.2003)

Date of mailing of the international search report

Authorized officer

Deborah C. Lambkin

Telephone No. 703-308-1235

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US

Commissioner for Patents

P.O. Box 1450

Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record.

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.